UNIVERSITY OF SAN DIEGO

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Genetic variation of coastal and offshore bottlenose dolphins, *Tursiops truncatus*, in the eastern North Pacific Ocean

A thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science in Marine Science

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2006

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ABSTRACT

Common bottlenose dolphins (*Tursiops truncatus*) are found worldwide in temperate and tropical regions, often with coastal and offshore forms. In the western North Atlantic Ocean, along the east coast of the United States, the coastal and offshore ecotypes have been distinguished based on morphology, hematology, parasite load, diet, habitat, photo-identification, and in some locations, genetic analysis. On the basis of morphological and photo-identification studies conducted on bottlenose dolphins in the eastern North Pacific Ocean, along the west coast of the U.S., two separate stocks have been designated for management: a coastal stock, estimated at about 300 individuals, and an offshore stock of 3,000 animals. This study is the first to analyze genetic differentiation between the coastal and offshore ecotypes in the eastern North Pacific Ocean.

A total of sixty-nine animals were biopsy sampled from coastal (located within 1 km of the shore, n = 29) and offshore dolphins (located greater than 4 km from the coast, n = 40). Both mitochondrial DNA (402 base pair sequence from the control region) and five microsatellite markers were examined. Coastal dolphins were found to have less genetic variability than offshore dolphins at both the nuclear and mitochondrial sites. Five haplotypes were identified for 29 coastal animals (gene diversity = 0.78 ± 0.04), while 25 haplotypes were identified for 40 offshore animals (gene diversity = 0.96 ± 0.02). There were no shared haplotypes between the two ecotypes. Gene diversity for the

microsatellite loci was 0.76 ± 0.43 for the offshore population (n = 38) and 0.54 ± 0.32 for the coastal population (n = 28).

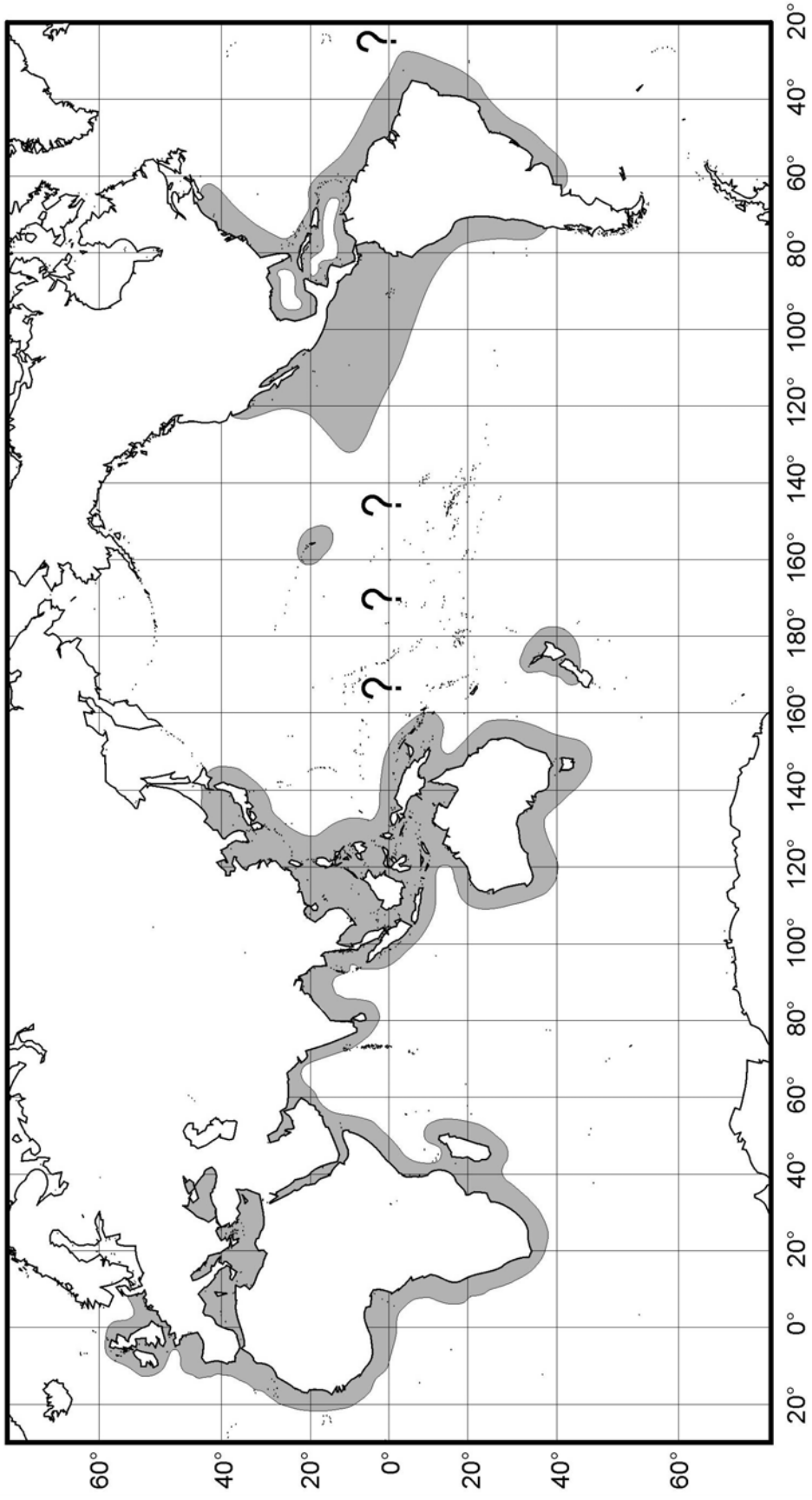
Significant genetic differentiation was found between the two populations for the mtDNA (Φ_{ST} = 0.27, $p < 0.005$) and microsatellite loci (F_{ST} = 0.22, $p < 0.005$). These results indicate strong differentiation that is consistent with long-term separation. This differentiation reinforces the decision to manage these ecotypes separately and to closely monitor the small coastal population, which is subject to anthropogenic threats and pollution.

CHAPTER 1: INTRODUCTION

Common bottlenose dolphins, *Tursiops truncatus*, are distributed worldwide in temperate and tropical regions (**Figure 1**), often with the existence of coastal and offshore forms (Ross 1977; Walker 1981; Duffield et al. 1983; Perrin 1984; Hersh and Duffield 1990; Mead and Potter 1990; Ross and Cockcroft 1990; Mead and Potter 1995; Curry 1997; Hoelzel et al. 1998b; Natoli et al. 2004; Segura García 2004; Carretta et al. 2005; Natoli et al. 2005; Sanino et al. 2005). Physiological and morphological variation within the distribution of bottlenose dolphins have resulted in numerous nominal species named within the *Tursiops* genus yet with limited supporting data (Walker 1981; Hoelzel et al. 1998b). Thus, researchers have recommended two accepted species, *Tursiops truncatus* and *Tursiops aduncus*, until further regional studies of variation are conducted (Walker 1981; Ross and Cockcroft 1990; Curry 1997; LeDuc et al. 1999; Wang et al. 1999; Möller and Beheregaray 2001; Wells and Scott 2002). The common bottlenose dolphin, *Tursiops truncatus*, is found in all temperate and tropical ocean basins and peripheral seas, while the Indo-Pacific bottlenose dolphin, *Tursiops aduncus*, inhabits the Indian and western Pacific Oceans (Rice 1998; Wells and Scott 2002).

Characteristics used to distinguish between the coastal and offshore forms are body size and skull morphology (Walker 1981; Hersh and Duffield 1990; Mead and Potter 1995), parasite load (Walker 1981; Mead and Potter 1995), diet (Walker 1981; Mead and Potter 1995), hematology analysis (Duffield et al. 1983), and distribution (Carretta et al. 2005). Coastal and offshore bottlenose

Figure 1: Global distribution of *Tursiops truncatus* is represented by the shaded region of the map (redrawn from Jefferson et al. 1993). Question marks indicate areas where distribution is not known.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dolphin forms have been identified in the waters of the United Kingdom, continental Europe, United States, Mexico, South Africa, South America, and Australia (Ross 1977; Walker 1981; Ross and Cockcroft 1990; Curry 1997; Hoelzel et al. 1998b; Möller and Beheregaray 2001; Parsons et al. 2002; Krützen et al. 2004; Natoli et al. 2004; Segura García 2004; Natoli et al. 2005; Sanino et al. 2005).

Although photo-identification studies provide information about dolphin distribution, behavior, occurrence, and range (e.g. Defran & Weller 1999), a study incorporating genetics can provide further information on phylogenetic and phylogeographical relationships (Curry and Smith 1997). Within the global distribution of *T. truncatus*, genetic studies have been conducted to examine population structure and molecular variation between and within local coastal and offshore populations for conservation and management purposes, as well as taxonomic clarification (Curry 1997; Curry and Smith 1997; Hoelzel et al. 1998b; Krützen et al. 2004; Natoli et al. 2004; Segura García 2004; Natoli et al. 2005; Sanino et al. 2005).

Along the coasts of the western North Atlantic (WNA) and northern Gulf of Mexico, there are multiple putative coastal populations consisting of resident, migrant, and transient groups (Scott 1990; Dowling and Brown 1993; Curry and Smith 1997; Hoelzel et al. 1998b; Barco et al. 1999). Genetic studies from these areas have shown the coastal and offshore forms to be genetically distinct, as well as, the coastal animals from the WNA and northern Gulf of Mexico to be genetically distinct (Dowling and Brown 1993; Curry 1997; Hoelzel et al. 1998b).

Both Curry (1997) and Hoelzel et al. (1998b) found the coastal dolphins to have lower genetic variation than the offshore dolphins in this region.

In the eastern North Pacific (ENP), morphological, photo-identification, and aerial surveys have identified a coastal and offshore form of bottlenose dolphin (Walker 1981; Defran and Weller 1999; Carretta et al. 2005). The coastal animals are estimated to number 323 individuals (95% CI = 259-430, Dudzik et al. in press) while the offshore animals are estimated at approximately 3,000 individuals within the United States Exclusive Economic Zone (EEZ, Carretta et al. 2005).

Coastal dolphins in the ENP region range from at least Ensenada, Mexico in the south to Monterey Bay, California in the north and are located within one kilometer of the shoreline (Defran et al. 1999; Carretta et al. 2005). These coastal animals are highly mobile within a narrow 'coastal corridor,' showing no site fidelity or pronounced patterns of seasonal occurrence (Defran and Weller 1999; Defran et al. 1999). Boat-based photo-identification surveys conducted along the coast within the Southern California Bight (SCB) showed high proportions of photographed coastal dolphins along Santa Barbara and Orange County to match photo-identified coastal dolphins along the San Diego coast (Santa Barbara = 88% and Orange County = 92 %, Defran et al. 1999).

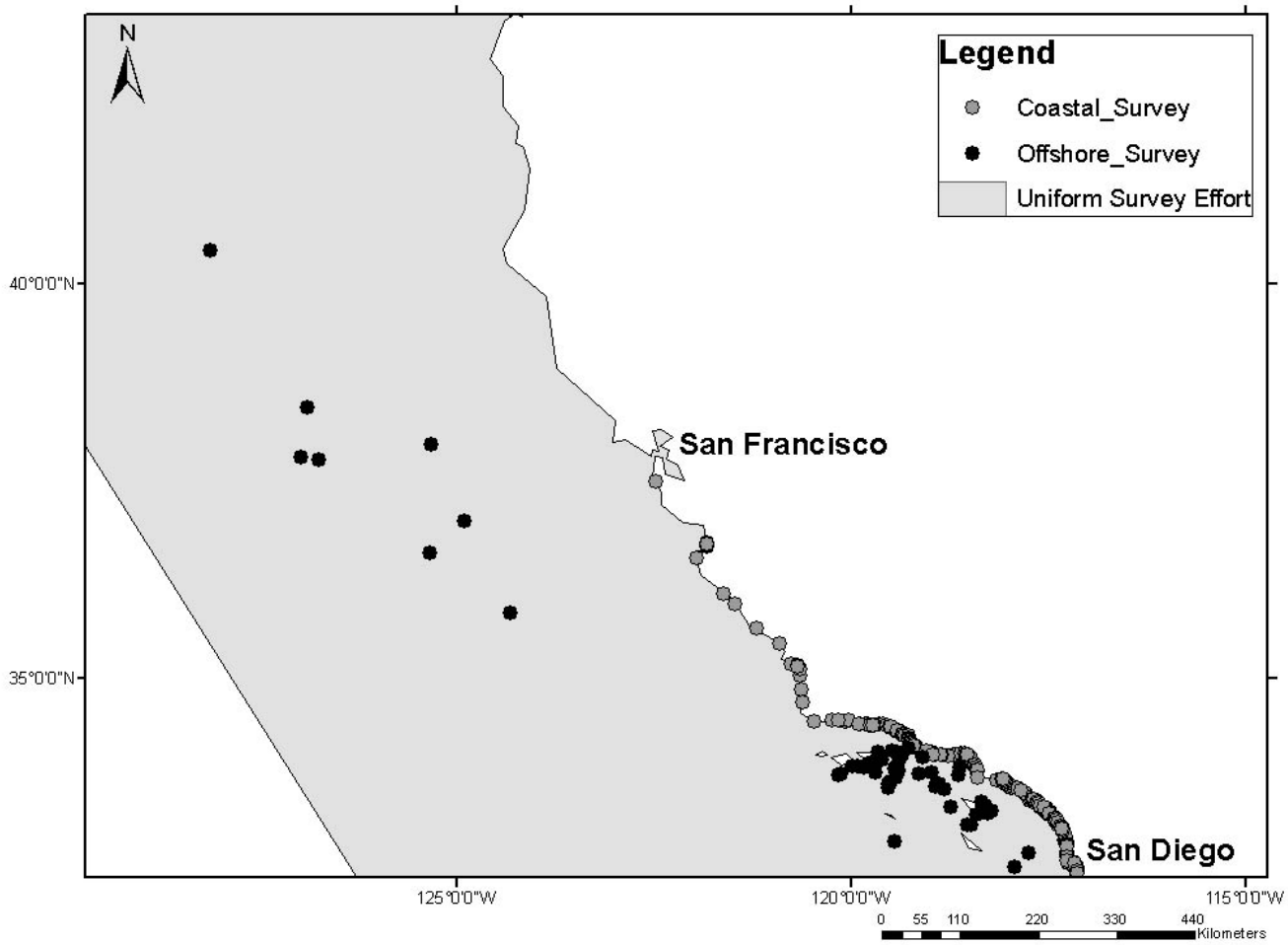
Offshore dolphins are located more than a kilometer from the coast throughout the U.S. EEZ, as far north as Oregon and Washington and well into the Eastern Tropical Pacific (ETP) to the south (Wells et al. 1990; Carretta et al. 2005). Surveys of offshore animals conducted around Santa Catalina Island, as

well as the offshore areas of San Pedro Channel and Palos Verdes Peninsula, found no photographically identified offshore individuals to match identified coastal individuals within the SCB (Schultz et al. 1988; DeDecker et al. 1999). The absence of photo-id matching between the animals of the coastal and pelagic areas suggests the two forms to be seemingly parapatric, however, it is not known whether there is gene flow between them (**Figure 2**, Hansen 1990).

For successful conservation and management of a species, it is important to determine population boundaries and genetic variability both within and between populations (Curry 1997). Genetic variability within a population increases its chance of adapting to a changing environment (Dizon 2002). Human encroachment and pollution are among some of the threats posed to bottlenose dolphins globally (Curry 1997). The coastal animals are particularly susceptible to anthropogenic threats, including pollutants and effluent from the coastline, putting them at risk for disease and die-off (Hansen 1990; Curry 1997).

This study examines the genetic differentiation between the coastal and offshore forms of bottlenose dolphins located within the ENP region, particularly along the west coast of the United States, to assess whether they are genetically distinct populations. By mitochondrial and nuclear DNA analysis, the genetic diversity and phylogenetic relationship of *Tursiops truncatus* in this area is compared to neighboring geographic locations within the species distribution. This regional study contributes new information on local genetic variation of the

Figure 2: Sightings of *T. truncatus* in the ENP, off the west coast of the United States. Coastal sightings are from aerial surveys and offshore sightings are from NOAA research cruise surveys (see Appendix II for data source). The gray region represents relatively uniform survey effort and the white area represents no survey effort.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Coastal_Survey
Offshore_Survey
Uniform Survey Effort
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35°0'0"N
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San Diego
125°0'0"W
120°0'0"W
115°0'0"W
0 55 110 220 330 440
Kilometers

nominal species to the ongoing debate of speciation within the genus *Tursiops* (Ross and Cockcroft 1990; Hoelzel et al. 1998b).

CHAPTER 2: LITERATURE REVIEW

Bottlenose dolphins (*Tursiops* sp.)

Common bottlenose dolphins, *Tursiops truncatus*, are found worldwide in temperate and tropical waters. They inhabit coastal and pelagic areas, as well as bays, lagoons, and estuaries (Hansen 1990; Curry and Smith 1997; Wells and Scott 2002; Krützen et al. 2004; Segura García 2004; Bearzi 2005). Morphological and physiological variation in these dolphins, correlated with differences in habitats, has caused much debate concerning the phylogeographical and phylogenetic relationships within this genus (Curry and Smith 1997).

Taxonomy

The geographical variations in morphotype have led to as many as 20 different species named within the genus *Tursiops* (Hoelzel et al. 1998b). Many of these species, however, were introduced on the basis of very limited data (Walker 1981). Researchers currently recognize two species within the *Tursiops* genus: *T. truncatus* and *T. aduncus* (Walker 1981; Ross and Cockcroft 1990; Curry 1997; Rice 1998; LeDuc et al. 1999; Wang et al. 1999; Möller and Beheregaray 2001). *Tursiops truncatus* (Montagu, 1821) is the common bottlenose dolphin found in coastal and offshore waters of tropical and temperate zones of all oceans and peripheral seas (Rice 1998; Wells and Scott 2002). *Tursiops aduncus* (Ehrenberg, 1833), the Indo-Pacific bottlenose dolphin, is a recognized coastal bottlenose dolphin in the Indian and western Pacific Oceans (Rice 1998; Wells and Scott 2002).

Tursiops truncatus

Common bottlenose dolphins in the western North Atlantic (WNA) waters have been referred to as *T. truncatus,* although morphological and physiological differences have been discovered between populations (Hersh and Duffield 1990; Mead and Potter 1990; Hoelzel et al. 1998b). In the eastern North Pacific Ocean (ENP), two species of bottlenose dolphins have been described: *T. gillii* (Dall) 1873 and *T. nuuanu* (Andrews) 1911 (Walker 1981). Walker (1981) referred to *T. gillii* as the coastal form and *T. nuuanu* as the offshore form. Hansen (1990), however, reported that *T. gillii* is considered by most researchers to be synonymous with *T. truncatus*. In fact, Tomilin (1957) (as cited by Curry 1997) and Mitchell (1975) (as cited by Curry 1997) further recommended that just one species of common bottlenose dolphin (*T. truncatus*) be considered until more detailed regional studies of variation are investigated in local *Tursiops* populations (Walker 1981; Ross and Cockcroft 1990).

Tursiops aduncus

T. aduncus was originally proposed as a new species in the southeast coastal waters of South Africa by Ross (1977) based on skull morphology and body size. This species is typically characterized by its coastal location, morphology, and ventral spotting (Rice 1998; Wang et al. 1999). The synonymous species found in the Red Sea, Queensland, Australia, and the Bay of Bengal was originally identified as *T. abusalam* (Ruppell 1842), *T. catalania* Gray (1862), and *T. gadamu* (Gray 1866), respectively (Rice 1998). However, based on a morphometric study of bottlenose dolphins on the east and west coasts

of Australia, Ross and Cockcroft (1990) decided that the morphological differences that led to the proposal of the *aduncus* species may be due to latitudinal variation. Therefore, they concluded the dolphins should be treated as a single species, *T. truncatus*. Yet, Wang et al. (1999), LeDuc et al. (1999), and Möller and Beheregaray (2001) later presented molecular evidence for the existence of *T. aduncus* in the Indo-Pacific and western South Pacific regions. Phylogenetic analysis further revealed that *T. aduncus* may not be a sister species to *T. truncatus,* but rather more closely related to the striped dolphin, *Stenella frontalis* (LeDuc et al. 1999).

Distribution

Tursiops truncatus

Within the distribution range of common bottlenose dolphins, there are resident, migrant, and transient groups (Curry and Smith 1997; Barco et al. 1999). Typically, animals that inhabit bays, lagoons, or estuaries tend to have high site fidelity or migrate seasonally to the area (Mead and Potter 1995; Curry 1997; Curry and Smith 1997). Observational studies have provided evidence of natal philopatry in bay populations (Curry and Smith 1997; Krützen et al. 2004). In other regions, common bottlenose dolphins have a more extensive distribution range with flexible habitat boundaries (Curry and Smith 1997; Defran and Weller 1999).

In United States waters, where there have been numerous studies done on the behavior, ecology, morphology, and distribution of bottlenose dolphins, *T. truncatus* populations are recognized in the WNA, northern Gulf of Mexico, and

ENP waters (Walker 1981; Curry 1997; Curry and Smith 1997; Hoelzel et al. 1998b; Carretta et al. 2005). In the western Atlantic Ocean, common bottlenose dolphins have been documented to extend from the north coast of Argentina to as far north as Nova Scotia during summer months (Kenney 1990; Wells and Scott 2002). Along the coasts of both the WNA and northern Gulf of Mexico, there seem to be multiple populations (Dowling and Brown 1993; Curry and Smith 1997; Hoelzel et al. 1998b). Scott (1990) found many local or resident populations in these coastal areas, in addition to migratory populations.

In the ENP along the western U.S., common bottlenose dolphins range along the California coast as far north as Monterey Bay and occasionally San Francisco, with sporadic pelagic sightings off Oregon and Washington during warm water periods (Wells et al. 1990; Carretta et al. 2005). Prior to the 1982-1983 El Niño, coastal dolphins in the Southern California Bight (SCB) were not observed farther north than Los Angeles County (Wells et al. 1990). However, the warm water brought northward by the El Niño increased primary productivity along north-central California, thus causing prey availability to be distributed further north (Defran et al. 1999). Since California coastal bottlenose dolphins do not have high site fidelity and will travel extensively along the coast in relation to food availability, the coastal dolphins extended their range to include the newly distributed prey (Defran et al. 1999). Interestingly, though the El Niño effects dissipated in subsequent years, the range of the coastal dolphins remained extended (Defran et al. 1999).

The fish species identified in the stomach contents of coastal dolphins are non-migratory, year-round inhabitants of the SCB, however, the coastal ecosystem of this region causes patchy and unpredictable distribution and abundance patterns of the prey (Defran et al. 1999). Thus, to locate the discontinuous prey resources, the dolphins travel long distances within their range (Defran et al. 1999).

Population differentiation within cetacean species is particularly difficult to determine due to their extensive ranges and highly mobile nature (Curry 1997). Photo-identification and observational studies can provide information about the species' behavior, distribution, occurrence, and range (Defran et al. 1999; Defran and Weller 1999).

The Cetacean Behavior Laboratory at San Diego State University, California investigated such factors on the California coastal bottlenose dolphins (*T. truncatus*) during a six year (January 1984 to December 1989) boat-based photo-identification survey along a 32 km coastline range extending from Scripps Pier, La Jolla north to South Carlsbad State Beach. Defran et al. (1999) and Defran and Weller (1999) identified animals in that region by comparing dorsal fin photographs of individual dolphins. By this mark-recapture method, it was discovered that the animals did not display long-term or seasonal site fidelity to the region but that the dolphins had a more extensive range than the allotted 32 km study area. Defran et al. (1999) found these dolphins to be highly mobile within a relatively narrow coastal corridor. This high mobility was suggested to be associated with variations in food resources. In another study, offshore

animals also did not show an apparent seasonality in distribution (Carretta et al. 2005).

An observational study done by Bearzi (2005) between 1997 and 2001 in Santa Monica Bay, California supported the results of Defran and Weller (1999) and Defran et al. (1999) that the coastal bottlenose dolphins in the SCB do not show long-term year-round site fidelity but are highly mobile. Defran et al. (1999) found eighty-eight percent of photographed coastal dolphins off Santa Barbara (43 of 49 identified dolphins) and ninety-two percent photographed off Orange County (123 of 133 identified dolphins) to match previously photo-identified coastal dolphins observed off of San Diego. The dolphins seem to have a large 'home range' in which they are opportunistic foragers (Bearzi 2005).

To determine the southern boundary of the coastal population observed in the SCB, Caldwell (1992) conducted a photo-identification study off San Quintín, Baja California Mexico. A previous study done off Ensenada, Mexico found eighty-eight percent of photos taken matched coastal animals identified from the San Diego area (60 of 68 identified dolphins; Caldwell 1992; Defran et al. 1999). Therefore, Defran et al. (1999) hypothesized the southernmost range limit may be further south than Ensenada. Caldwell (1992) found only one individual out of 105 identified dolphins (1.0 %) within her three months of survey that matched a sighted animal further north off California. Caldwell (1992) suggested the southern range boundary most likely lies between Ensenada and San Quintín and that a high degree of discreteness exists between the SCB and Mexico coastal bottlenose dolphin populations.

Tursiops aduncus

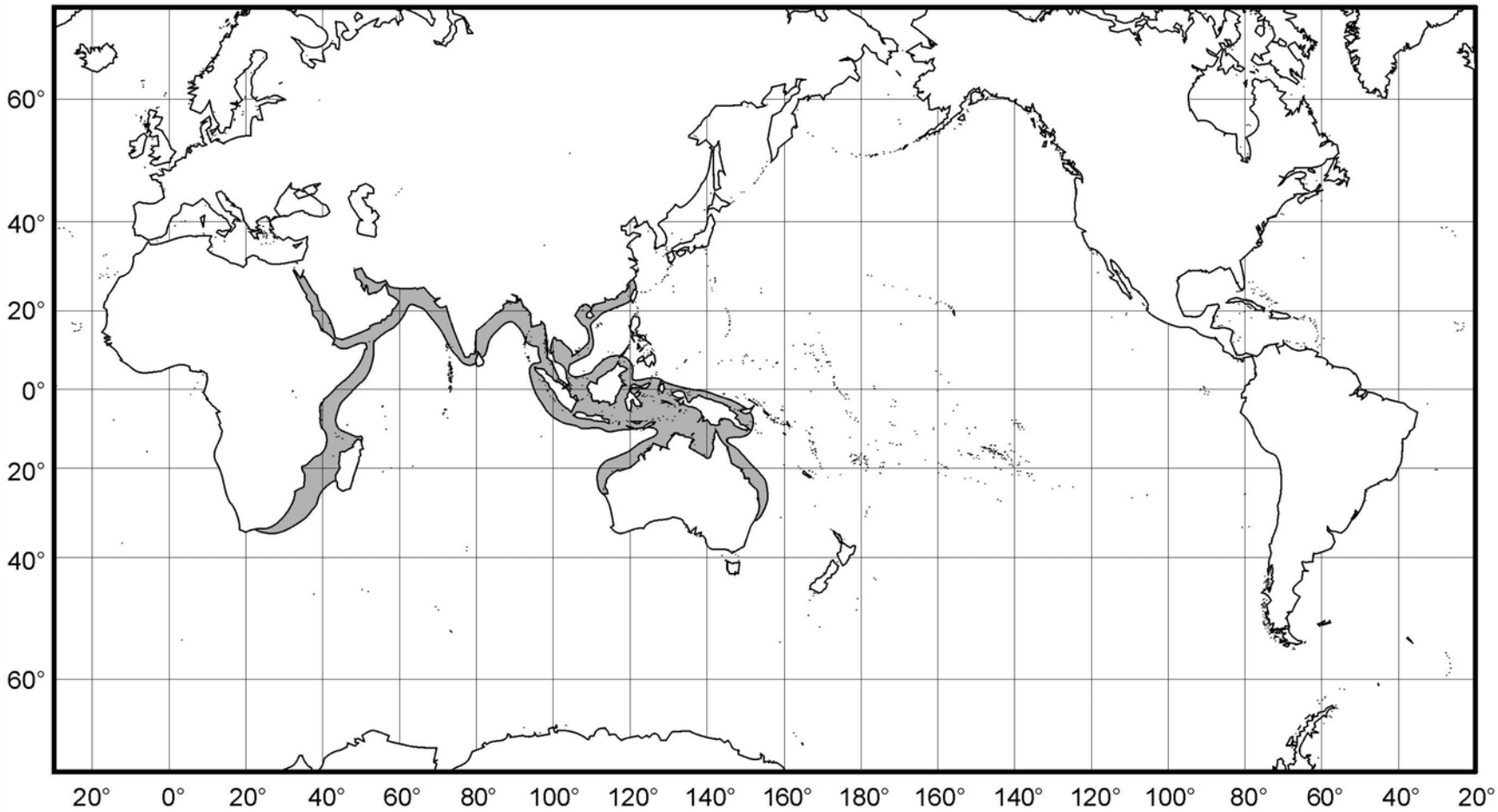
The Indo-Pacific bottlenose dolphin ranges along South Africa, north to the Red Sea, and eastward through the Persian Gulf, Arabian Sea, and the Bay of Bengal (Rice 1998). This seemingly coastal animal has also been identified along Taiwan, China, and Australia (**Figure 3**; Rice 1998; Wang et al. 1999; Möller and Beheregaray 2001; Wells and Scott 2002; Krützen et al. 2004).

Tursiops truncatus vs. *Tursiops aduncus*

Though the ranges of these two *Tursiops* sp. overlap along South Africa, Australia, and China, their morphology, distribution, and genetic distinctness support *T. truncatus* and *T. aduncus* to be separate species (Ross 1977; Ross and Cockcroft 1990; Curry 1997; Rice 1998; LeDuc et al. 1999; Wells and Scott 2002). As *T. truncatus* is found worldwide, excluding high latitudes, *T. aduncus* seems concentrated within the Indian and southwest Pacific Oceans (Curry 1997; Rice 1998; Wang et al. 1999; Möller and Beheregaray 2001; Krützen et al. 2004). Though there is a coastal and offshore ecotype of *T. truncatus* identified throughout its distribution, *T. aduncus* seems to only be found in coastal areas (Ross 1977; Ross and Cockcroft 1990; Rice 1998; Wang et al. 1999; Möller and Beheregaray 2001; Wells and Scott 2002).

T. aduncus is most commonly distinguished from *T. truncatus* by ventral and lateral spotting, an elongated beak, as well as cranial measurements and number of vertebrae (Ross 1977; Wang et al. 2000). Although there does seem to be latitudinal variation in body length, beak length, skull size, and ventral spotting within the species, as Ross and Cockcroft (1990) found variation in local coastal

Figure 3: Global distribution of *Tursiops aduncus* is represented by the shaded region on the map (redrawn from Jefferson et al. in prep).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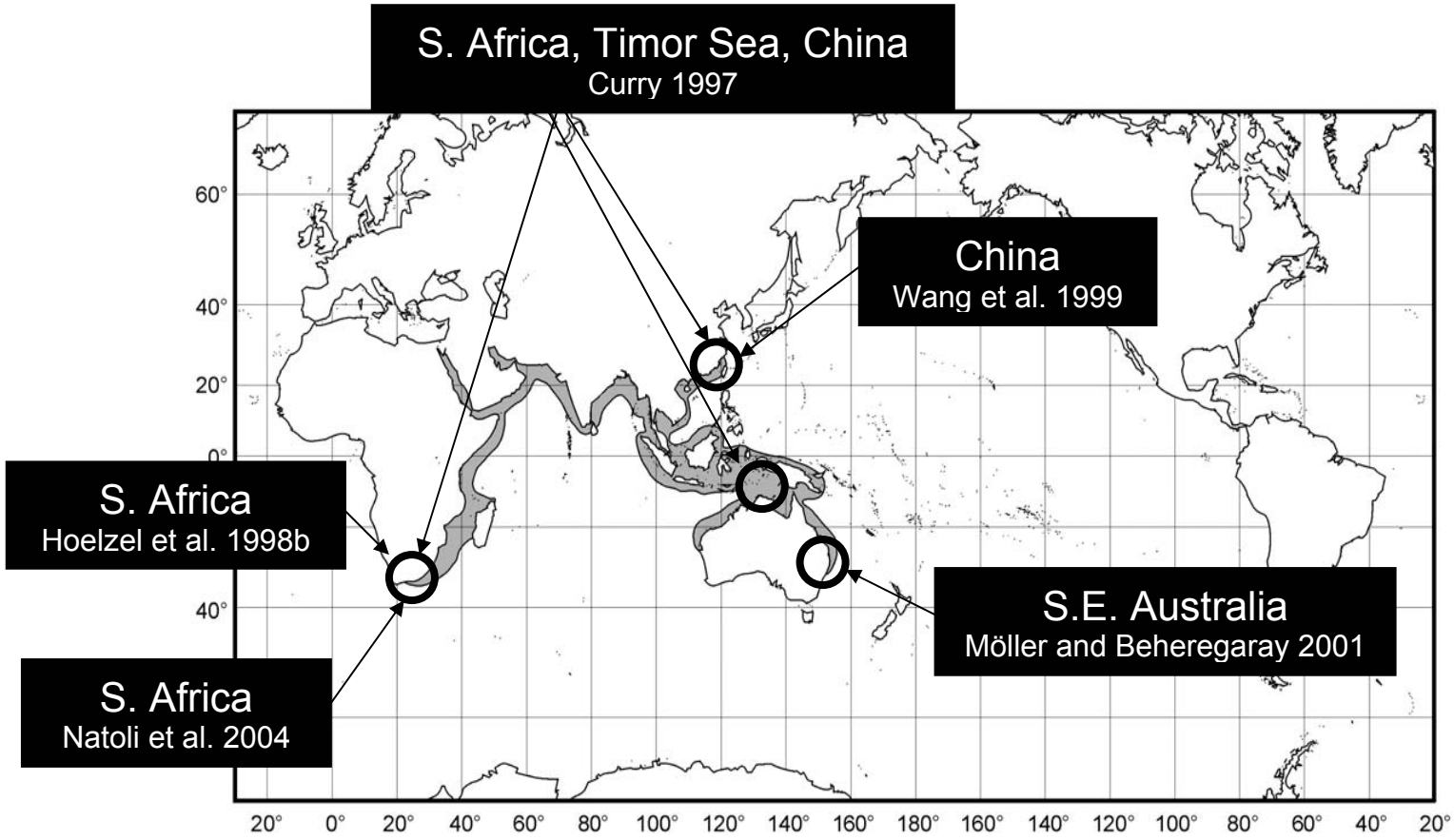
populations along Australia. Ventral spotting was characteristic of animals along the northern coast, but southern animals along Australia were unspotted (Ross and Cockcroft 1990; Möller and Beheregaray 2001). Spotted and unspotted animals were also observed in Chinese waters (Wang et al. 2000).

T. truncatus and *T. aduncus* have been distinguished by genetic analysis which is a vital tool in species level distinction as shown in the studies conducted by Henshaw et al. (1997) and LeDuc et al. (1999). Henshaw et al. (1997) used mitochondrial DNA (mtDNA), which is maternally inherited, to identify ten different species of beaked whales. By species-specific DNA patterns, the study could properly identify by-caught beaked whales by analyzing only a small sample of collected tissue instead of by labor intensive skull assessment. LeDuc et al. (1999) used mtDNA analysis to determine the phylogeny of several delphinid species previously classified by morphology. Although many of the results concurred with current taxonomy, the study did indicate a number of systematic revisions.

T. truncatus and *T. aduncus* have been found to be genetically distinct with fixed nucleotide differences between them (**Figure 4**; Curry 1997; LeDuc et al. 1999; Wang et al. 1999). Upon classifying two distinct forms of bottlenose dolphins in Chinese waters by morphological characteristics, Wang et al. (1999) used mtDNA analysis to confirm genetic distinction. The study identified two reproductively isolated *Tursiops* species: *T. truncatus* and *T. aduncus*, based on the finding of seven fixed site differences within the sequenced region. Möller and Beheregaray (2001) went on to use the findings of Wang et al. (1999) to

Figure 4: *Tursiops aduncus* genetic studies


S. Africa, Timor Sea, China
Curry 1997
China
Wang et al. 1999
S. Africa
Hoelzel et al. 1998b
S.E. Australia
Möller and Beheregaray 2001
S. Africa
Natoli et al. 2004
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determine that two coastal bottlenose dolphin populations along the east coast of Australia were comprised of *T. aduncus*, extending the range of the species to southwestern Pacific waters.

The phylogenetic analysis on delphinid species conducted by LeDuc et al. (1999) revealed *T. aduncus* to be more closely related to striped dolphin, *Stenella frontalis*, than the species *T. truncatus*. Natoli et al. (2004) found the *T. aduncus* species in the Indo-Pacific and South African waters to be highly differentiated not only from the *truncatus* species but also from each other, suggesting the two *aduncus* types to possibly be separate species. Since the bottlenose dolphins found in the ENP have been recognized as *Tursiops truncatus*, from here on within this thesis the term bottlenose dolphin will refer to *T. truncatus*.

Population subdivision in *T. truncatus:* coastal and offshore forms

The most common pattern of geographical variation in delphinids is the morphological distinction between coastal and offshore populations (Perrin 1984). In many parts of the world, both a coastal and an offshore form of bottlenose dolphin (*Tursiops truncatus*) have been recognized (Ross 1977; Walker 1981; Duffield et al. 1983; Hersh and Duffield 1990; Mead and Potter 1990; Ross and Cockcroft 1990; Mead and Potter 1995; Curry 1997; Hoelzel et al. 1998b; Natoli et al. 2004; Segura García 2004; Carretta et al. 2005; Natoli et al. 2005; Sanino et al. 2005). In some locations, the two ecotypes are sympatric populations while in others they are parapatric (Hansen 1990; Defran and Weller 1999; Torres et al. 2003; Segura García 2004; Carretta et al. 2005).

Torres et al. (2003) found that although the coastal and offshore bottlenose dolphins in the WNA, from New York to Florida, are sympatric in regions, all biopsy samples collected within 7.5 kilometers of shore were of coastal dolphins. They also discovered that samples collected beyond 34 km from shore and in water deeper than 34 m were all offshore animals. Offshore animals seem to concentrate along the continental shelf break, with scattered sightings beyond the shelf (Kenney 1990). Coastal animals show a more limited distribution that includes warmer and shallower waters than offshore animals (Kenney 1990). During winter months, coastal dolphins are not observed along the northeast U.S. in the WNA (Kenney 1990).

In the ENP, along the west coast of the United States, Defran and Weller (1999) and Carretta et al. (2005) documented the coastal animals to be found within 1 km from shore, where water depth ranges are typically from 10 to 30 m. These surveys also concluded that the offshore animals, located greater than 1 km from the shoreline, are parapatric to the coastal animals.

Both coastal and offshore forms in the WNA have seasonal migrants, extending their geographic range to a maximum during summer months when water temperature is warmer (Mead and Potter 1990; Barco et al. 1999). The coastal animals consist of permanent residents, transients, and migrants (Barco et al. 1999). In the winter, dolphins are rarely seen north of Cape Hatteras, North Carolina, but in summer, the migrants travel north and are seen along the Virginia, Maryland, and Delaware coasts (Mead and Potter 1990; Barco et al. 1999).

The two forms of *T. truncatus* differ in body size, skull morphology, feeding habits, parasite load, and hematology (Walker 1981; Duffield et al. 1983; Hersh and Duffield 1990; Mead and Potter 1990). Along the WNA and northern Gulf of Mexico (Curry 1997), the coastal animals are found to be smaller in body length than the offshore animals. But in the ENP and Gulf of California, Walker (1981) documented the coastal form as larger than the offshore bottlenose dolphins.

In spotted dolphins (*Stenella attenuata*), Schnell et al. (1986) hypothesized that the variation in body length was influenced by environmental factors, namely water temperature. However Ross and Cockcroft (1990) did not find a correlation between water temperature and differences in body size of coastal and offshore bottlenose dolphins in southern Queensland, Australia.

The difference in size may also relate to habitat differences. Hersh and Duffield (1990) proposed that the smaller coastal form may be an adaptation for more maneuverability in shallow environments. They also surmised that the smaller body size may be thermally disadvantageous in cooler waters. This hypothesis would explain why coastal dolphins in the ENP are larger than the offshore animals since parts of the coastline are upwelling regions of cooler water (Hersh and Duffield 1990).

Skull morphology also differs between the two forms of dolphins (Walker 1981; Ross and Cockcroft 1990; Mead and Potter 1995; Hoelzel et al. 1998b). Walker (1981) measured the condylobasal length (CBL is the length from the rostrum to the occipital condyl) and the shape of the rostrum of ENP bottlenose

dolphins and found that the offshore animals had a smaller mean CBL and a more slender, tapering rostrum than the coastal animals. Ross and Cockcroft (1990) also measured the CBL of coastal and offshore animals from the Australian coast and found smaller skulls to correlate with lower latitudes. In the WNA, Mead and Potter (1995) found relatively little variance in the skull morphology of the offshore form, while the coastal form exhibited enough variance to suggest derivation from multiple populations. Hoelzel et al. (1998b) also found offshore bottlenose dolphins in the WNA had wider nasal bones than the coastal animals.

The two forms of dolphins feed on different prey because they inhabit areas of different depths (Walker 1981; Mead and Potter 1995; Hoelzel et al. 1998b). By examining stomach contents of stranded and by-caught dolphins, Walker (1981) discovered that coastal dolphins in the ENP, particularly off the California coast, feed primarily on fishes and invertebrates of the littoral and sublittoral zones. Since he did not have any samples of offshore animals off California, he compared his finding to ETP offshore bottlenose dolphins that were found to feed primarily on epipelagic fish and cephalopods. Similar findings were discovered for the WNA dolphins, where Hoelzel et al. (1998b) identified coastal species of fish and cephalopods in the stomachs of coastal bottlenose dolphins, while offshore dolphins had pelagic species of fish and squid.

The differences observed in parasite load, likely related to differences in diet, between coastal and offshore animals can be used as a natural biological tag for population identification (Walker 1981). Walker (1981) and Mead and Potter (1995) found the three common parasite species (*Phyllobothrium*, *Monorhygma*,

and *Crassicauda*) that infect offshore dolphins in the WNA and ENP Oceans were not detected in the coastal dolphins of those regions. Similarly, the brauninid trematode, *Braunina cordiformis*, was found in coastal animals but not in offshore dolphins (Mead and Potter 1990). Van Waerebeek et al. (1990) found the same to be true in coastal and offshore bottlenose dolphins from the eastern South Pacific off the South American coast.

Hersh and Duffield (1990) found the offshore animals of the WNA to have a higher hemoglobin (Hb) concentration, hematocrit, and red blood cell count than the coastal animals. They also discovered the offshore animals to have two electrophoretically distinguishable hemoglobins while the coastal animals had one. Coastal animals had only the electrophoretically fast hemoglobin, which comprised about 30% of the offshore animals' hemoglobin (Hersh and Duffield 1990).

Small odontocetes which exhibit different activity level, habitat, and diving capacity have been shown to differ in Hb concentration, blood volume, and packed cell volume (Duffield et al. 1983). In comparing a deep-diving, fast swimming offshore Dall's porpoise (*Phocoenoides dalli*), an offshore Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), and a coastal Atlantic bottlenose dolphin (*Tursiops truncatus*), Duffield et al. (1983) found the hemoglobin concentration to decrease respectively. The researchers attributed the higher hemoglobin concentration, hematocrits, and count of red blood cells to facilitate cetaceans in deep dives by having greater oxygen-carrying capacity. This increased respiratory function correlates with Mead and Potter's (1995) and

Hoelzel et al.'s (1998b) discovery of a greater nareal diameter in offshore bottlenose dolphins relative to coastal dolphins.

Population Genetics

Genetic studies that are management-oriented focus on mitochondrial DNA (mtDNA) and microsatellites in the nuclear DNA (Dizon 2002). Mitochondrial DNA can provide information on genetic differentiation within and between populations because it is inherited from the mother and has a fast rate of sequence evolution (Maldonado et al. 1995). By sequencing the mtDNA nucleotides, the maternal lineages and population subdivision within a cetacean species can be detected with high resolution (Brown Gladden et al. 1997). The sequenced mtDNA region is compared among individuals within and between populations, with unique sequences being termed haplotypes (Dizon 2002). In studies where genetic differentiation is found among the mtDNA sequences, further analysis of the nuclear diversity is needed to confirm the pattern, often involving microsatellites (Wang et al. 1999; Parsons et al. 2002).

Microsatellites are short stretches of repeated DNA within the nuclear genome and show exceptional variability in most species (Dizon 2002). Studies have analyzed five or more microsatellite loci within the DNA to characterize the level of allele sharing within a genus (Hoelzel et al. 1998b; Rooney et al. 1999; Möller and Beheregaray 2001). The allele sizes recorded for each microsatellite locus are designated as the individual's genotype (Dizon 2002). Microsatellites are used to determine paternity and genetic diversity which can be used to

interpret numerous behavioral and ecological characteristics of a cetacean species (Shinohara et al. 1997; Rooney et al. 1999).

One constraint on genetic studies is obtaining the tissue sample for analysis. Many delphinid studies use DNA samples extracted from either stranded or by-caught animals, thus the true origin of the animal is unknown and may bias the results (Curry 1997; Walton 1997; Natoli et al. 2004; Natoli et al. 2005).

Cetacean population genetics

Numerous studies focused on discerning the population structure within a species have included genetic analysis (Richard et al. 1996; Brown Gladden et al. 1997; Curry 1997; Henshaw et al. 1997; O'Corry-Crowe 1997; Hoelzel et al. 1998b; Wang et al. 1999; Möller and Beheregaray 2001; Krützen et al. 2004; Natoli et al. 2004; Natoli et al. 2005; Sanino et al. 2005). Richard et al. (1996) examined mtDNA and microsatellite DNA in sperm whales (*Physeter macrocephalus*) off the coast of Ecuador to find that though groups were composed of multiple matrilines there was paternal relatedness among the individuals.

Brown Gladden et al. (1997) and O'Corry-Crowe et al. (1997) used mtDNA sequence variation to investigate population structure of beluga whales (*Delphinapterus leucas*) along North America. Even in the absence of geographical barriers, the animals' strong philopatry to specific summering areas resulted in genetic differentiation among populations, the extent of which needs to be determined by nuclear DNA analysis (Brown Gladden et al. 1997).

Delphinid population genetics

In the Eastern Tropical Pacific (ETP), a coastal and offshore subspecies of spotted dolphins, *Stenella attenuata*, have been distinguished by body size, coloration/spotting, and morphology (Schnell et al. 1982; Perrin et al. 1994). Escorza-Treviño et al. (2005) investigated genetic differentiation between the coastal and offshore spotted dolphin populations by mtDNA and microsatellite DNA analysis. The study found shared haplotypes between the forms and low nucleotide diversity, suggesting either continued gene flow or interrupted gene flow with insufficient time for lineage sorting to occur. The study also revealed significant structure within the previously recognized single panmictic coastal population, an important result for the conservation and management of the subspecies.

Rosel et al. (1994) genetically distinguished two species within the genus *Delphinus*. The sympatric populations of short-beaked and long-beaked forms of common dolphins were already known to be distinct in color pattern, external morphology, and cranial characteristics (Heyning and Perrin 1994). By analyzing the control region and cytochrome B gene of mtDNA, Rosel et al. (1994) not only found no shared haplotypes between the two morphotypes but two fixed nucleotide substitutions. The genetic and morphological differentiation suggested no gene flow between the two forms and supported the existence of two species that should be managed separately (Rosel et al. 1994).

Walton (1997) studied the population structure of harbor porpoises (*Phocoena phocoena*) in the seas around the United Kingdom and adjacent waters

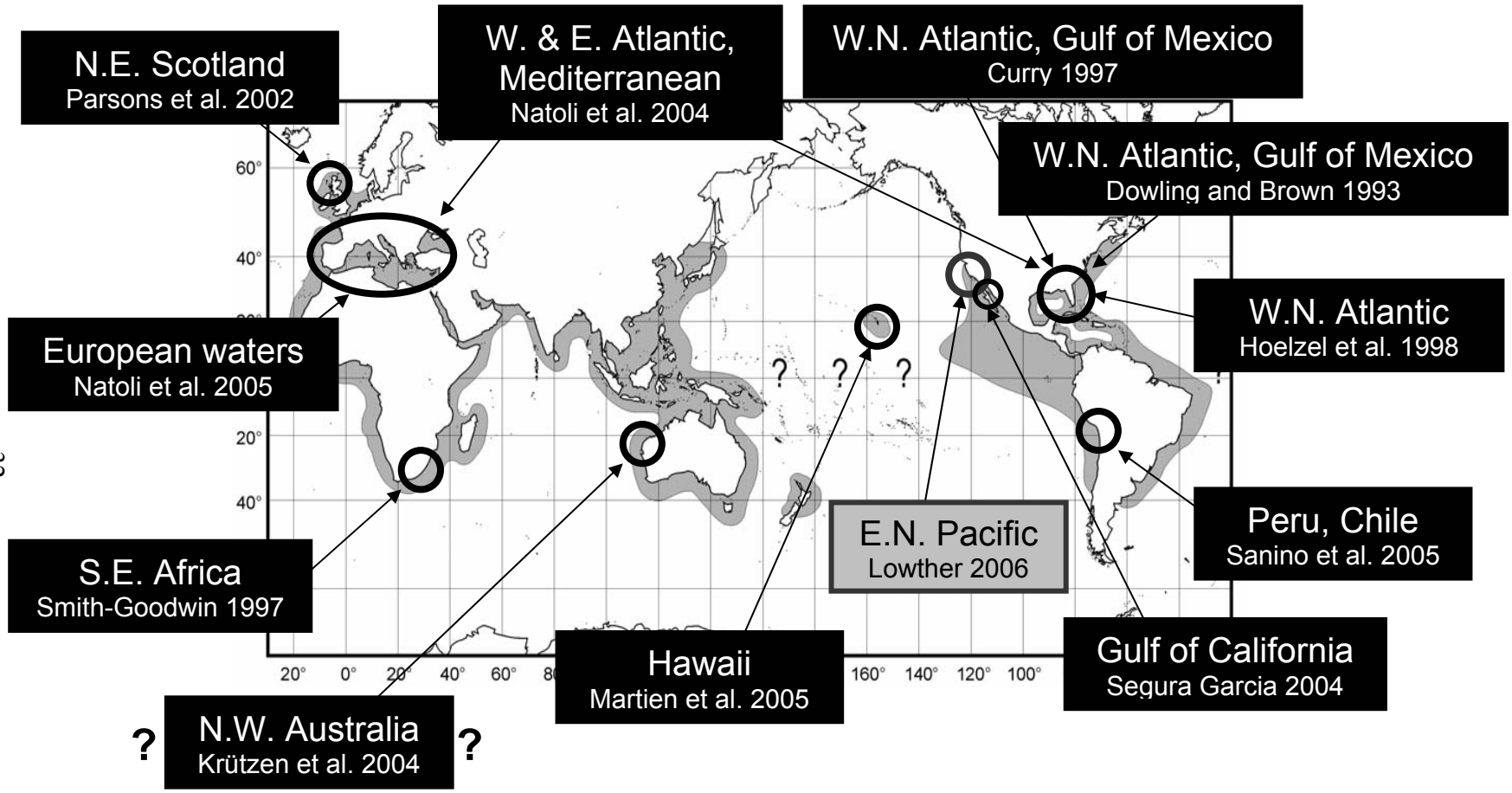
to better assess the effects of incidental by-caught deaths on the species in that region. By examining mtDNA variability for isolated populations, the study found some degree of geographical sub-structuring attributed to female philopatry. Thus, Walton (1997) suggested a serious depletion of animals in one area may not result in replenishment from animals of a neighboring region.

Bottlenose dolphin population genetics

Genetic studies on bottlenose dolphins have focused on population structure and speciation in the genus *Tursiops* with emphasis on coastal and offshore populations (**Figure 5**; Curry 1997; Hoelzel et al. 1998b; Wang et al. 1999; Möller and Beheregaray 2001; Krützen et al. 2004; Natoli et al. 2004; Segura García 2004; Natoli et al. 2005; Sanino et al. 2005). Coastal populations have been documented globally to have lower genetic variation, lower allelic richness and hetereozygosity than offshore populations (Curry 1997; Hoelzel et al. 1998b; Krützen et al. 2004; Natoli et al. 2004; Segura García 2004; Natoli et al. 2005; Sanino et al. 2005). It is thought that the more diverse pelagic population may represent the source for independent coastal founder events (Natoli et al. 2004). The reduced diversity found in coastal populations may also be a result of historical bottlenecks or demographic cycles (Natoli et al. 2004).

By using samples collected worldwide, Natoli et al. (2004) discovered significant genetic diversity and differentiation among populations defined by geographic region or habitat. The study found pelagic populations had higher genetic diversity and allelic richness than coastal populations. A minimum

Figure 5: *Tursiops truncatus* genetic studies that have been conducted worldwide. The current study is indicated in gray while studies that have been done are indicated in black. The Krützen et al. (2004) study is marked with question marks because it is uncertain which species of *Tursiops* the study referred to in that area.


N.E. Scotland
Parsons et al. 2002
W. & E. Atlantic, Mediterranean
Natoli et al. 2004
W.N. Atlantic, Gulf of Mexico
Curry 1997
W.N. Atlantic, Gulf of Mexico
Dowling and Brown 1993
W.N. Atlantic
Hoelzel et al. 1998
European waters
Natoli et al. 2005
E.N. Pacific
Lowther 2006
Peru, Chile
Sanino et al. 2005
S.E. Africa
Smith-Goodwin 1997
Hawaii
Martien et al. 2005
Gulf of California
Segura Garcia 2004
? N.W. Australia ?
Krützen et al. 2004
60°
40°
20°
40°
20°
0°
20°
40°
60°
160°
140°
120°
100°

spanning network showed genetic relatedness of haplotypes from coastal dolphins in the WNA, northern Gulf of Mexico, and the Bahamas to have separate exclusive branches from the offshore haplotypes of those regions (Natoli et al. 2004).

Krützen et al. (2004) investigated genetic diversity of coastal bottlenose dolphins inhabiting Shark Bay in Western Australia to assess population structure. These animals are referred to as *Tursiops* sp., because they have not yet been classified as *T. truncatus* or *T. aduncus*. Of the 220 individual biopsies they collected, the researchers discovered eight haplotypes. Krützen et al. (2004) suggested this low genetic diversity may be due to females having restricted dispersal, not venturing far from their natal area, which would result in significant genetic differentiation among non-adjacent localities.

A genetic study of a small sample of bottlenose dolphins from the southeast Pacific Ocean revealed three distinct reproductive units: two coastal and one offshore population (Sanino et al. 2005). Sanino et al. (2005) assessed the mitochondrial control region of 11 coastal and 20 offshore bottlenose dolphins off Peru and Chile to determine genetic diversity and phylogenetic relationships. The Chilean and Peruvian coastal populations were highly divergent, suggesting these populations to have high site fidelity, likely preventing them from mixing (Sanino et al. 2005). Although there was significant genetic differentiation between the Peru-Chile offshore population and Chilean coastals, the Chilean coastal population was more closely related to the offshore population than to the Peruvian coastal population (Sanino et al. 2005). Sanino et al. (2005) concluded

if the Peruvian coastal population is reproductively isolated, which needs to be confirmed by nuclear genetic analysis, the long-term survival of this small community of thirty individuals is uncertain and should be managed as an evolutionarily significant unit.

The status of the *T. truncatus* population in the coastal regions of the United Kingdom was examined after a survey revealed a decline in the North Sea population (Parsons et al. 2002). An increase in development and human activity along northeast Scotland led Parsons et al. (2002) to designate appropriate management units by assessing the genetic diversity within this dolphin population and its genetic relatedness to neighboring populations in the waters around the United Kingdom and Ireland. The study found bottlenose dolphins along northeast Scotland to be more closely related to dolphins along Wales than to the nearest geographic neighboring population along west Scotland. The analysis indicated that the northeast Scotland dolphins are geographically isolated from the surrounding bottlenose dolphin populations of the United Kingdom. The low level of genetic diversity (two mtDNA haplotypes found within 15 samples) found for the northeast Scotland population needs further nuclear diversity analysis to confirm the pattern of geographic isolation for successful management implications (Parsons et al. 2002).

Natoli et al. (2005) went on to compare the bottlenose dolphins from Scotland and UK waters to populations located in the eastern North Atlantic, western Mediterranean, eastern Mediterranean, and Black Sea. The study investigated patterns of gene flow across this geographic range that has large- and

fine-scale habitat structure, to see if physical oceanographic boundaries that define habitat regions lead to regional population structure in coastal *T. truncatus*.

By assessing mitochondrial and nuclear DNA markers, Natoli et al. (2005) found the highest genetic differentiation between the Black Sea animals and those of the other areas, while samples from the western Mediterranean and eastern North Atlantic had the lowest genetic differentiation. The minimum spanning network, depicting phylogeny, did not support lineage sorting of the lower genetic diversity Black Sea population from the other four putative populations, suggesting structure too recent to detect (Natoli et al. 2005). The researchers concluded that prey distribution, reflecting habitat differences, were defining the geographical range and association patterns of the local bottlenose dolphin populations (Natoli et al. 2005).

The coastal and offshore ecotypes identified in the Gulf of California have been reported by Walker (1981) to be synonymous with the Southern California coastal and ETP offshore animals. Although the two ecotypes are sympatric in some areas within the Gulf and do share haplotypes, Segura et al. (in prep) discovered that the coastal and offshore forms are not panmictic as there was significant genetic differentiation between them, signifying reduced gene flow. The genetic study showed the offshore animals to have a higher genetic diversity than the coastal animals, as has been found globally. Segura et al.'s (in prep) evaluation proposed the two ecotypes within the Gulf of California to be designated and managed as separate stocks.

Multiple stocks may also be proposed for *T. truncatus* in the Hawaiian Archipelago, where a photo-identification study has found a lack of movement of marked individuals among islands and a possible depth distribution of dolphins at two islands (Martien et al. 2005). Upon genetic analysis of mtDNA for 121 animals, Martien et al. (2005) discovered significant differences in haplotype frequencies among dolphins from the different islands and depth strata. The study suggested, upon further nuclear evaluation, the management stocks in this region be redefined.

The heavily researched bottlenose dolphins of the WNA are commonly used as a model of fine-scale population structure to compare population characteristics of *Tursiops truncatus* in other regions of its range (Curry 1997; Curry and Smith 1997; Hoelzel et al. 1998b; Natoli et al. 2004). In addition to the distinctness in morphology, parasite load, diet, and hemoglobin profile between the coastal and offshore populations of the WNA region, distinct genetic differentiation has also been found between the two forms (Dowling and Brown 1993; Curry 1997; Curry and Smith 1997; Hoelzel et al. 1998b).

Hoelzel et al. (1998b) found significant genetic differentiation in mitochondrial and nuclear DNA markers between the coastal and offshore bottlenose dolphins of the WNA. There was no overlap of mtDNA haplotypes between the populations (Hoelzel et al. 1998b). Curry (1997) further found two fixed nucleotide differences in the mtDNA sequence between the coastal and offshore animals. Both Hoelzel et al. (1998b) and Curry (1997) discovered

monophyly of the coastal population, suggesting sufficient isolation between the coastal and offshore populations for nucleotide sites to reach fixation.

The high degree of site fidelity exhibited by the coastal animals in this region contributes to lower dispersal between populations, thus causing less genetic variation in the coastal populations (Hoelzel et al. 1998b). Curry (1997) found coastal populations in the northern Gulf of Mexico to be significantly different in mtDNA diversity than the coastal population of the WNA. This finding had previously been introduced by restriction endonuclease analysis in the mtDNA (Dowling and Brown 1993). The genetic divergence between the WNA and northern Gulf of Mexico coastal populations is consistent with genetic data obtained from previous studies on a variety of coastal organisms (Dowling and Brown 1993). Dowling and Brown (1993) and Curry (1997) hypothesized the genetic separation occurring between the coastal bottlenose dolphins near the southern tip of Florida may be due to water current patterns forming a barrier in prey distribution, thus reducing gene flow between the dolphin populations.

Among the five microsatellite loci Hoelzel et al. (1998b) analyzed, alleles unique to coastal and offshore populations were detected, however, there was overlap of allelic frequency between the two populations. The greater allelic diversity was found in the offshore population suggesting a possibly high level of dispersal among offshore animals (Hoelzel et al. 1998b). Overall, the clear genetic distinction between the coastal and offshore populations found by Hoelzel et al. (1998b) and Curry (1997), combined with previous morphological and

ecological differences between the, in some locations, sympatric populations, support the hypothesis that they are separate species.

Hoelzel et al. (1998b), however, could not find as clear a genetic distinction between the coastal and offshore populations of southern Africa as was found in the WNA. In fact, shared mtDNA haplotypes were found between coastal and offshore African *T. truncatus* animals, suggesting continued gene flow between the two forms or a recent separation that cannot yet be detected. Resident coastal populations may exist along southeastern Africa as Smith-Goodwin (1997) discovered low genetic variation in putative resident bottlenose dolphin populations displaying regional philopatry.

In the ENP, the extent of gene flow is not known between the coastal and offshore *T. truncatus* forms. Though the two forms have been differentiated based on morphology, parasite load, diet, and habitat (Walker 1981; Defran and Weller 1999; Carretta et al. 2005), a genetic analysis had not been conducted until the current study.

Globally, coastal bottlenose dolphins have lower genetic variation and allelic richness than offshore bottlenose dolphins (Curry 1997; Hoelzel et al. 1998b; Natoli et al. 2004). In the coastal vs. offshore study done by Hoelzel et al. (1998b) in the WNA, there were no shared haplotypes between the two forms, thus supporting genetic distinctness. In the Indo-Pacific region, the coastal animals have actually been identified as a separate species from *T. truncatus* (Wang et al. 1999; Möller and Beheregaray 2001). There is still much debate and lack of clarity concerning the population structure and speciation of *Tursiops*,

however further genetic and morphological studies will aid in deciphering their taxonomic status (Curry and Smith 1997).

Population management

In order to successfully conserve and manage a cetacean species, it is important to determine population boundaries and whether there is gene flow across those boundaries (Dizon 2002). Lande (1991) asserted that the initial population boundaries of cetacean species should be determined by morphology, behavior, and geographic distribution. He said these characteristics, which are used in classical systematics, can imply reproductively isolated populations. Therefore, one population cannot be replenished from another. Lande (1991) also mentioned that molecular genetics can be used to distinguish sibling species and subspecies.

By finding differentiation between coastal and offshore bottlenose dolphin forms, the degree of harmful threats posed to each form can be assessed for conservation implications (Curry 1997). In some locations where bottlenose dolphins are found there is little conservation legislation by developing nations to successfully manage the species (Wang et al. 1999). Mortality is a concern for dolphins by-caught in the commercial fishing industry particularly in coastal gillnets (Torres et al. 2003).

The coastal animals are more vulnerable to harmful threats since they inhabit the coastal region where human encroachment and pollution are more abundant (Curry 1997; Wang et al. 1999). Anthropogenic factors are believed to influence the severity of viral outbreaks in marine mammal populations (Hall et

al. 1992). Coastal bottlenose dolphins in the ENP are documented to have the highest level of DDT concentration of all wild marine mammals, which could influence their reproductive rate (Hansen 1990). Thus, it is important to determine if interbreeding is occurring between the coastal and offshore animals of the eastern North Pacific region.

CHAPTER 3: METHODS

Sample Collection

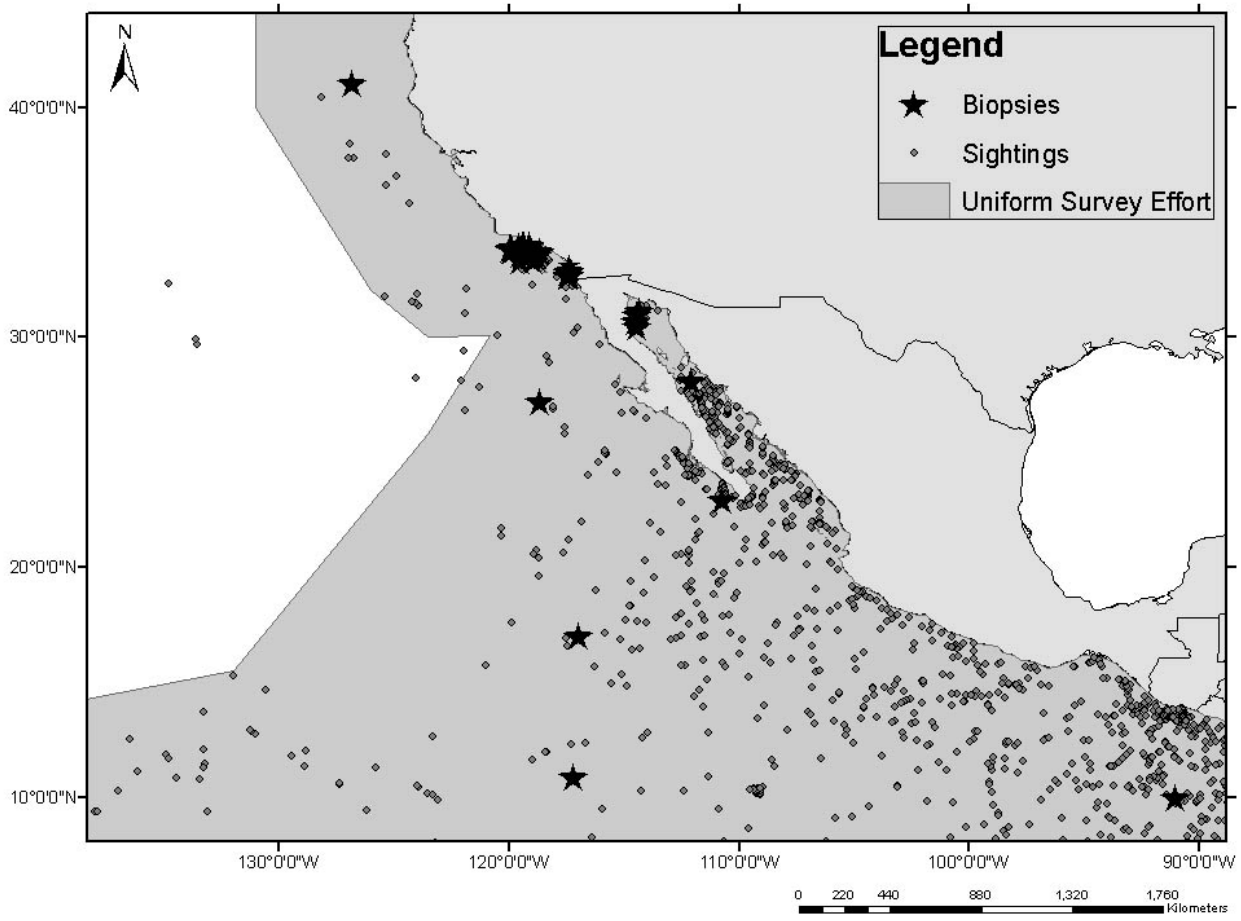
Skin biopsy samples were collected from 77 common bottlenose dolphins (Appendix I) in the ENP, off the western United States, in winter (December – February), summer (June - August), and fall (September – November) seasons between 1992 and 2005 (**Table 1**). There was no sampling effort in the spring season, though dolphins were observed (Dudzik et al. in press). Samples were obtained in the SCB by NOAA research cruises and small boat sampling effort. One sample was collected from a by-caught dolphin in the pelagic waters off Oregon. A small number of biopsy samples collected from the ETP and the Gulf of California (Gulf-CA) were also included in this study for comparison of overall genetic variation within the ENP region and determination of population subdivision (**Figure 6**).

A Barnett Wildcat III crossbow (draw weight 150 lbs) delivering a carbon biopsy arrow with modified tip was used to collect biopsy samples. The tip was 25 mm in length with a 7 mm diameter round end and contained three internal prongs to retain the tissue sample composed of skin and blubber that, in most cases, measured approximately one centimeter in length. In order to prevent infection, each tip was disinfected in 10 % bleach, scrubbed with a test tube brush, and rinsed in 70 % ethanol. All samples were archived in the Southwest Fisheries Science Center (SWFSC) tissue collection and stored in 20 % salt saturated dimethyl sulfoxide (DMSO) solution, ethanol, or frozen dry. DMSO and ethanol samples were stored at -20 °C while frozen dry samples were stored

Table 1: Sampling distribution of *T. truncatus* biopsy samples by season and year. No sampling effort occurred in spring months.

	Season							
Year	***Winter***		***Spring***		***Summer***		***Fall***	
	Coastal	*Offshore*	*Coastal*	*Offshore*	*Coastal*	*Offshore*	*Coastal*	*Offshore*
1992	-	-	-	-	-	-	-	**2**
1995	-	-	-	-	-	**5**	-	-
1996	-	-	-	-	-	**6**	-	**3**
2000	-	**6**	-	-	-	-	-	-
2001	-	-	-	-	**1**	**4**	**2**	**15**
2002	-	-	-	-	-	-	-	**1**
2004	-	-	-	-	**8**	**3**	**6**	-
2005	**14**	-	-	-	-	-	**1**	-

Figure 6: *T. truncatus* sightings and biopsy locations within the eastern North Pacific Ocean. Gray dots represent sightings (see Appendix II for data source) and black stars are locations of biopsies used in this study (see Appendix I). The shaded gray area represents relatively uniform survey effort while the white area in the ENP represents sparse survey effort.

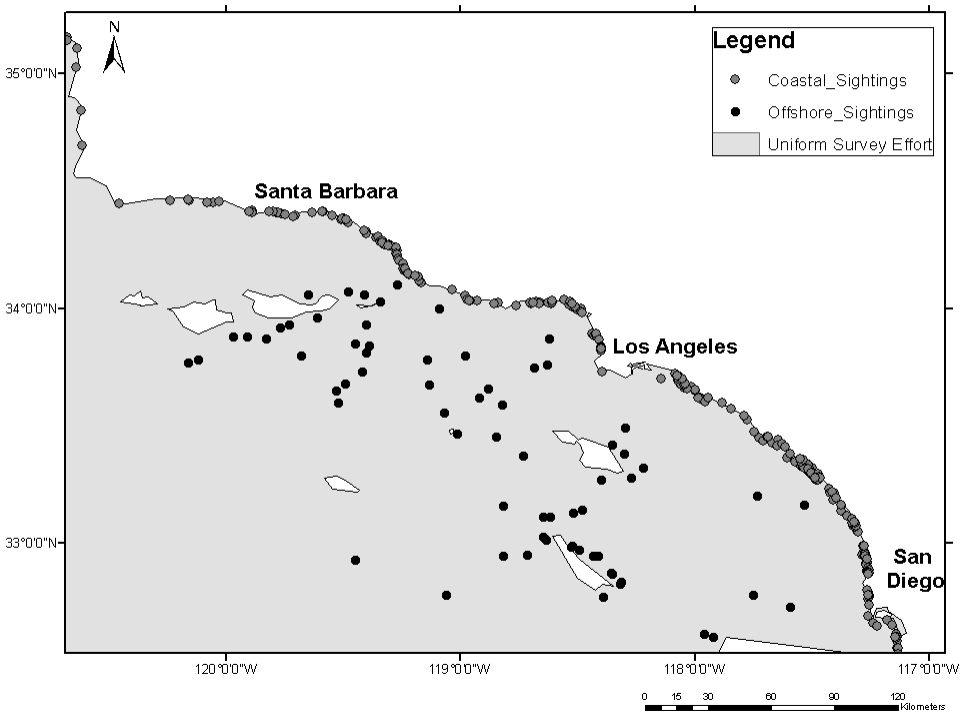

N
Legend
Biopsies
Sightings
Uniform Survey Effort
40°0'0"N
30°0'0"N
20°0'0"N
10°0'0"N
130°0'0"W
120°0'0"W
110°0'0"W
100°0'0"W
90°0'0"W
0 220 440 880 1,320 1,760
Kilometers

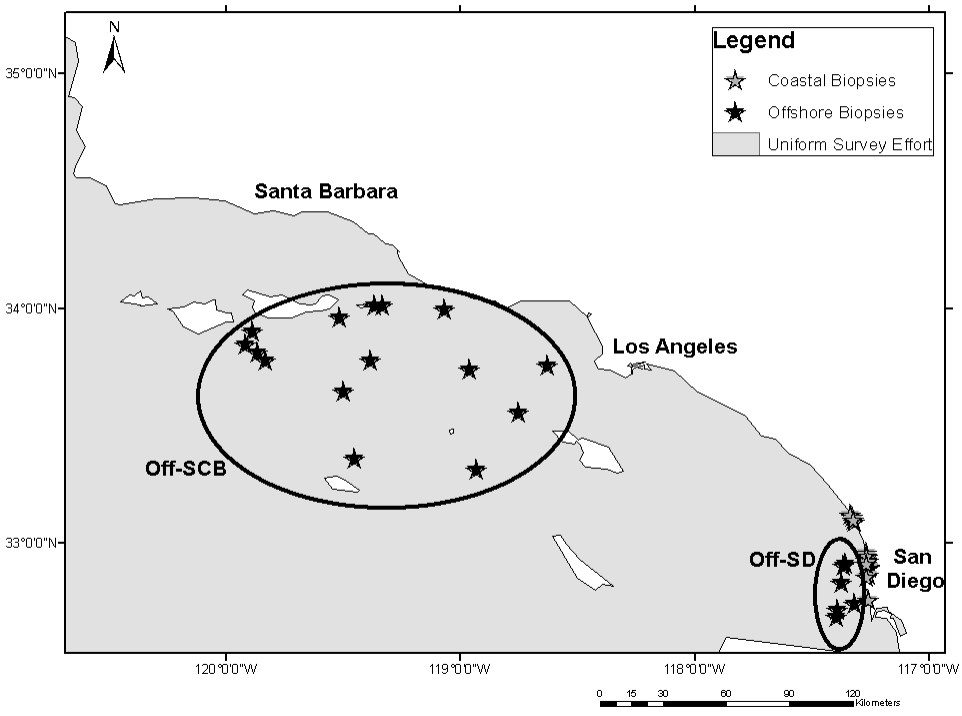
at -80 °C.

Assignment of the samples to the two ecotypes, coastal and offshore, was determined *a-priori* by biopsy sample location. Aerial surveys, observational, and photo-identification studies have determined that the coastal ecotype remains within one kilometer of the shore and travels freely within this relatively restricted corridor from Ensenada, Mexico to Monterey Bay and occasionally San Francisco, California (Hansen 1990; Defran and Weller 1999; Defran et al. 1999; Carretta et al. 2005). All 31 coastal dolphin samples were collected within one kilometer of the San Diego county coastline, between Oceanside and Mission Bay. The offshore ecotype is distributed greater than four kilometers from the coastline throughout the SCB and pelagic waters of the ENP off the western United States and Mexico (Defran and Weller 1999; Carretta et al. 2005). Forty-four of the offshore dolphin samples came from the SCB and one was collected from an offshore by-caught dolphin off Oregon.

The distribution of offshore biopsy locations and offshore bottlenose dolphin sightings in the ENP indicated possible stratification among the offshore samples. Therefore, the offshore samples were divided into a northern and southern group, respectively: offshore Southern California Bight (Off-SCB) and offshore San Diego (Off-SD) (**Figure 7**). The Off-SCB group was composed of samples collected between the Channel Islands during NOAA research cruises (see Appendix II for data source), in addition to the one by-caught animal off Oregon. The Off-SD group was composed of biopsies collected greater than

Figure 7: Sightings and biopsy locations of *T. truncatus* within the Southern California Bight. The top map shows distribution of sightings for coastal and offshore dolphins. The bottom map shows biopsy locations of coastal and offshore samples used in this study. The offshore biopsies were grouped into Off-SCB and Off-SD as indicated on the map. The Off-SCB also included one sample from the pelagic waters off Oregon, not shown on map. Biopsy and sighting data sources are referenced in Appendix I and II, respectively. The shaded gray region represents area of relatively uniform survey effort.


N
Legend
Coastal_Sightings
Offshore_Sightings
Uniform Survey Effort
Santa Barbara
Los Angeles
San Diego
35°0'0"N
34°0'0"N
33°0'0"N
120°0'0"W
119°0'0"W
118°0'0"W
117°0'0"W
0 15 30 60 90 120
Kilometers


N
Legend
Coastal Biopsies
Offshore Biopsies
Uniform Survey Effort
Santa Barbara
Los Angeles
Off-SCB
Off-SD
San Diego
35°0'0"N
34°0'0"N
33°0'0"N
120°0'0"W
119°0'0"W
118°0'0"W
117°0'0"W
0 15 30 60 90 120
Kilometers

4 km from the San Diego county coastline and south of the Channel Islands.

Molecular methods

DNA extraction and genetic sexing

DNA was extracted using standard molecular protocols (Qiagen DNeasy, Palumbi et al. 1991; Saiki et al. 1988). Older samples that initially yielded a low level of DNA were re-extracted by a sodium chloride protein precipitation method (protocol available on request from SWFSC) then cleaned with the DNeasy protocol. The DNA yield was assessed by spectrophotometer.

Samples were genetically sexed by amplification of DNA from the zinc finger gene region (X-chromosome) and sex determining region (Y-chromosome) (Fain and LeMay 1995). PCR product was electrophoresed through 2 % agarose stained with ethidium bromide, and visualized on an UV transilluminator. Females were identified by a single band (X chromosome) while males revealed two bands (X and Y chromosomes). Sex results were then verified using a technique of double-labelled fluorescent probes in a MX3000P Real-Time PCR System (Stratagene) on the zinc finger (ZFX and ZFY) genes (Morin et al. 2005).

mtDNA amplification and sequencing

A 402 base pair segment of the mitochondrial control region (D-loop) was amplified by polymerase chain reaction (PCR) using primers D (5' – cctgaagtaagaaccagatg - 3'; Rosel et al. 1994) and Tro (5' – cctccctaagactcaagg – 3'; developed at SWFSC). Standard PCR protocols were used: 50 µl reactions containing 2 µl of DNA and 48 µl of mix consisting of 36.75 µl of MilliQ water (Millipore, Bedford, MA), 5 µl of 10x PCR buffer (500 mM KCl, 100 mM Tris-

HCl, pH 8.3, and 15 mM $MgCl_2$), 3 µl of 10 mM dNTP, 1.5 µl of each 10 mM primer, and 2.5 units of Taq DNA polymerase. For PCR cycling, the thermal cycler was set at 90 °C for 2.5 min, then 35 cycles of 94 °C for 45 sec, 48 °C for 1 min, and 72 °C for 1.5 min, followed by an extension at 72 °C for 5 min. The amplified PCR product was then cleaned using QIAquick 250 (Qiagen).

Sequencing was performed using 12 µl reactions of 3-4 µl of cleaned PCR product (based on concentration of product), 3-4 µl of MilliQ water (adjusted to the amount of PCR product used), 3 µl of 1 µM primer, and 2 µl of Big Dye (ABI version 1.1). The cycle sequencing reaction parameters were 30 cycles at 96 °C for 10 sec, 50 °C for 5 sec, and 60 °C for 4 min. Both strands of the amplified DNA product were sequenced separately as mutual controls on the Applied Biosystems Inc. (ABI) model 3100 sequencer using standard protocols. Sequences were aligned by eye using Sequencher v4.1 software (Gene Codes Corp., 2000). Haplotypes were assigned using HapFinder software (F.I. Archer, personal communication).

Microsatellite genotyping

Microsatellite DNA primers for five loci (dinucleotide repeats) were analyzed for 64 samples. Primer sets for loci KWM2a and KWM12a were derived from killer whales (Hoelzel et al. 1998a), locus EV37 from humpback whales (Hoelzel et al. 2002), and TexVet7 and D8 were derived from bottlenose dolphins (Rooney et al. 1999 and Shinohara et al. 1997, respectively). Extracted DNA was amplified using a 25 µl reaction of 1 µl of DNA, 18 µl of MilliQ water, 2.5 µl of 10x PCR buffer (same as sequencing buffer), 1.5 µl of 10 mM dNTP,

0.75 µl of each 10 µM primer, and 2.5 units of Taq DNA polymerase. The PCR cycling profile consisted of 90 °C for 2.5 min, followed by 35 cycles of 94 °C for 45 sec, 1 min at annealing temperature, and 72 °C for 1.5 min, then a final extension of 72 °C for 5 min. The optimal annealing temperature was 55 °C for D8, EV37, and TexVet7 and 45 °C for KWM2a and KWM12a.

Purity and size of amplification were assessed electrophoretically on a 2 % agarose gel before loading onto the ABI 3100 Genetic Analyzer. ABI GENESCAN was used along with an internal standard marker, Genescan-500 ROX, Applied Biosystems Inc., to determine allele fragment size. Allelic frequency per population was assessed using analysis MS-toolkit (http://oscar.gen.tcd.ie/~sdepark/ms-toolkit/).

Data analysis

Data editing

Individuals that matched in sex, mtDNA haplotype, and microsatellite genotype were deemed duplicate samples and one copy was discarded from the sample set. The two insertion/deletion (indel) sites found in the mtDNA control region sequenced were not used as variable sites in analysis.

Genetic diversity analysis

Arlequin 2.0.1.1 software (Schneider et al. 2000) was used to calculate the minimum spanning network and AMOVA for genetic diversity. Gene diversity ($\hat{H}$) was estimated by the following equation:

$$\hat{H} = \frac{n}{n-1}(1 - \sum_{i=1}^{k} p_i^2)$$

where n is the number of gene copies in the sample, k is the number of haplotypes, and p_i is the sample frequency of the i-th haplotype (Nei 1987).

The minimum spanning network provided a visual interpretation of the relatedness among haplotypes both within and between populations. The genetic diversity indicated the probability of randomly choosing two different haplotypes within a population. Polymorphic sites were assessed using MEGA version 3.1 software (Kumar et al. 2004).

Population subdivision analysis

Arlequin 2.0.1.1 software (Schneider et al. 2000) was used to run AMOVA, population comparison, Φst, F_{ST}, and Hardy-Weinberg Equilibrium analysis. The Φst values from AMOVA are a measure of differentiation in haplotype variation between populations taking into account genetic distance among haplotypes and haplotype frequency (Excoffier et al. 1992). The F_{ST} value from AMOVA provided the variation of allelic frequency distribution that was attributed to between population variance differences. Pairwise comparisons were run for Φst and F_{ST} statistics to determine genetic differentiation among putative populations. Hardy-Weinberg Equilibrium analysis indicated whether individuals within a population were randomly mating or if further population structure was evident.

Chi-square analysis was used to examine differences in haplotype frequency between populations (http://swfsc.nmfs.noaa.gov/PRD/PROGRAMS/POP-ID/default.htm). The Monte Carlo p-value as described in Roff and Bentzen (1989), derived from random

permutations of the data, was used in this study and ran with 10,000 iterations. Linkage disequilibrium for all pairs of loci in each population were assessed using Fisher's exact test and Markov chain method in GenePop 3.4d (Raymond and Rousset 1995). Structure v.2 was used to assess population structure by a model-based clustering method using genotype data (Pritchard et al. 2000).

Additional Gulf of California sequences

Eighty-six sequences from the Gulf of California (Gulf-CA) coastal and offshore bottlenose dolphin ecotypes published in the thesis work of Segura García (2004) were comparable to the sequences of the current study and hence were incorporated in additional analyses to provide a more complete study of the Gulf region. Based on the designation of coastal and offshore ecotypes as reported by Segura García (2004) and Segura et al. (in prep), the seven Gulf-CA samples from the current study were assigned to the appropriate form.

Six samples from the current study were duplicates of individuals used in Segura García's (2004) study. For duplicate samples, the sequence from the current study was used in analyses and the duplicate published sequence was discarded from the sample set. One offshore sample of the current study was added to the offshore sequences from Segura García (2004). In total, there were 34 coastal sequences, designated as Gulf-CA coastal, and 53 offshore sequences, designated as Gulf-CA offshore. Without access to the original tissue, the sequences from Segura García (2004) could not be verified but were included in analyses outside of the primary focus of the current study on coastal and offshore

bottlenose dolphins in the ENP. Analyses incorporating these data are reported at the end of the results section.

CHAPTER 4: RESULTS

Samples Collected

A total of 77 biopsy samples were collected of coastal and offshore common bottlenose dolphins in the ENP region, off the western United States. Ten biopsy samples from the ETP and seven biopsy samples from the Gulf of California were also included in the sample set to provide an indication of genetic variation and population structure for the ENP region.

Duplicates

Samples that matched in sex, mtDNA haplotype, and microsatellite genotype were determined to be duplicates. Two duplicate samples were discovered among the offshore samples and three among the coastal samples. All but one of the duplicates were incidences where an individual animal was sampled twice within the same sampling event of a group. The remaining duplicate was of a coastal animal biopsied twice within four years. All duplicates were males. Once the duplicate samples were removed, the final sample sizes were 40 offshore animals and 29 coastal animals (Appendix I).

Sex

Samples from the offshore dolphins were not significantly different from a 1:1 sex ratio distribution (52 % male, 48 % female, $p = 0.32$). However, samples from the coastal dolphins were 68 % male and 32 % female, which was significantly different from a 1:1 sex ratio ($p = 0.018$). This bias towards males indicated either the true sex ratio among the animals was not 1:1 or that sampling was not random. It is possible the biopsy crew came upon male dominant groups,

as male alliances have been reported for bottlenose dolphins (Wells et al. 1987 as cited by Parsons et al. 2003). Male and female dolphins both are documented to avoid small boats (Lusseau 2003).

Genetic diversity

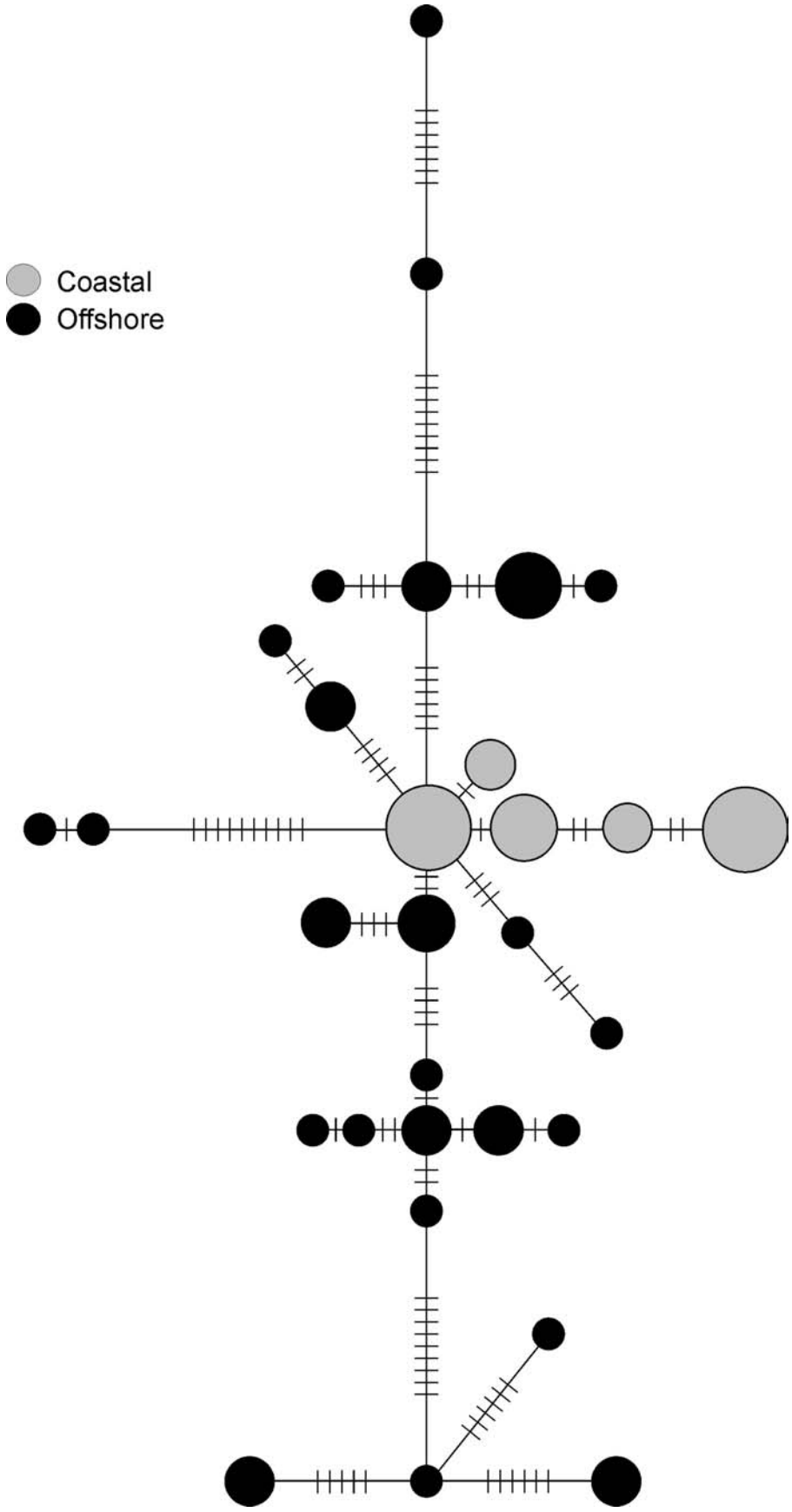
There were 37 variable sites discovered within the 30 coastal and offshore haplotypes (**Table 2**). Five haplotypes with five variable sites were identified for the 29 coastal animals, while 25 haplotypes with 36 variable sites were identified for the 40 offshore animals. The most common haplotype among the offshore dolphins (haplotype # 8) was present in seven individuals. Haplotype # 27 and # 29 were the most common haplotypes found among the coastal dolphins with nine individuals representing each, respectively. There were no shared haplotypes between the coastal and offshore forms. There were two indels, at site 7 and site 129, in the Off-SCB samples and one indel (site 7) in the Off-SD samples. No indels were found in the coastal samples.

The minimum spanning network of the coastal and offshore haplotypes depicted a coastal haplotype (# 27) as the center haplotype with the most branches (seven) stemming from it (**Figure 8**). The coastal samples had two exclusive branches with small genetic distance between haplotypes, one to two base pairs apart. The offshore samples had a greater diversity of haplotypes and larger between haplotype distance than the coastal samples. The closest offshore haplotype to the central coastal haplotype was two to three base pairs away.

Table 2: Polymorphic sites and haplotype frequencies for the 402 base pairs sequenced within the mitochondrial D-loop control region for samples from each of the five areas (Off-SCB, Off-SD, Coastal, ETP, and Gulf-CA). Haplotype number is indicated in the first column.

	Polymorphic Sites				Haplotype Frequencies				
	111111 022399000125 725449138192	1112222222 5580456777 3458818257	2222222222 7888888999 8012356067	3333333333 0003677999 5682036347	Off-SCB N=22	Off-SD N=18	Coastal N=29	ETP N=10	Gulf-CA N=7
1	-GATATTTAT-C	ATTCCGCCAC	ATTTTCCATC	CATTCCTATC	1				4
2	-.....C...-.	....	....C...C.	...C.T....	2				1
3	-..CG.C..CTT	....TA....	...C......	...C.T....	2				
4	-.....C...-.	...T..T...	.....T...T	...C.T.GC.	1				
5	-..C.CC..C-T	...TT..T..	..CC.T....	.G.C.T....	1	1			
6	-.....C...-.	...TT.T...	.....T....	...C...GC.	2				
7	-.....CC..-.	....	........C.	...C......	3				1
8	-.....C...-.	...T..T...	.....T...T	...C...GC.	4	3			
9	-A...CC.G.-T	.C....A.G.	...C.T....	.G.C......	1				
10	-.........-.	....	.........T	.....T..C.		1			
11	-........C-.	.......T..	G........T	....		1			
12	A.....C...-.	......T...	.....TT.CT	.G.C..C...		1		1	
13	-.........-.	....	.........T	........C.		2			
14	-.........-.	....	.........T	....	1	1			
15	A....CC...-T	.........T	G.CC......	.G.C....C.		1			
16	-.....C...-.	......T...	.......GCT	.G.C..C...		1			
17	-.....C...-.	......T...	.........CT	...CT.C...		1			
18	-.........-.	....	.........T	...C.T....		1			
19	A.....C...-.	......T...	.....T..CT	.G.C......		2			1
20	-..C..C..C-T	...T......	..CC.....T	T..C.T..C.	1				
21	A.....C...-T	....T....A	..CC.T....	...C....CT	1				
22	-..C..C..C-T	G...T.....	..CC......	...C.T....	1				
23	-A...CC.G.-T	.C....A...	...C.T....	.G.C......	1				
24	-........C-.	....	G........T	....		1			
25	-.....C...-.	...T..T...	.....T....	.G.....GC.		1			
26	-.....C...-.	......TT..	........C.	.G.C.T....			3		
27	-.....C...-.	......T...	........C.	...C......			9		
28	-.....C...-.	......TT..	........C.	...C......			6		
29	-.....C...-.	......T...	......T.C.	.G.C.T....			9		
30	-.....C...-.	......T...	........C.	..CC......			2		
31	-A...CC.G.-T	......A...	...C.T....	.G.C......				3	
32	A.G...C...-.	......T...	.....T..CT	.G.C..C...				1	
33	-.....C...-.	....	.........T	...C......				1	
34	-.....C.G.-.	...T..T...	.....T...T	...C...GC.				1	
35	-.........-.	....	.........T	...C......				1	
36	A.....C...-.	......T...	.....T..CT	.G.C..C...				1	
37	-.....C...-.	..CT..T...	.C...T....	...C...GC.				1	

Figure 8: Minimum spanning network of haplotypes for the coastal and offshore forms in the ENP, along the western United States. Gray circles represent coastal haplotypes and black circles represent offshore haplotypes. Each circle represents a haplotype with the size of the circle relative to the number of individuals sequenced with that haplotype, ranging from 1 to 9 individuals. The ticks on each branch represent the number of base pair differences between haplotypes.


Coastal
Offshore

Population subdivision coastal vs. offshore

The χ^2-test indicated a significant difference in haplotype frequency between the coastal and offshore forms ($\chi^2 = 68.0$, df = 29, Monte Carlo $p < 0.005$). The significant difference in variation between the forms indicated by the AMOVA suggested evidence for population structure ($\Phi_{ST} = 0.27$, $p < 0.005$).

The coastal ecotype had substantially fewer alleles at each of the five microsatellite loci analyzed relative to the offshore ecotype (**Table 3**). The polymorphic sites ranged from 7 to 19 alleles in the offshore dolphins and 3 to 4 alleles in the coastal dolphins. Of the 43 unique alleles detected, 41 were found in the offshore form. The two unique alleles found among the coastal form, at loci KWM2a and KWM12a, were at relatively high frequencies, 25 % and 23 %, respectively (**Figure 9**).

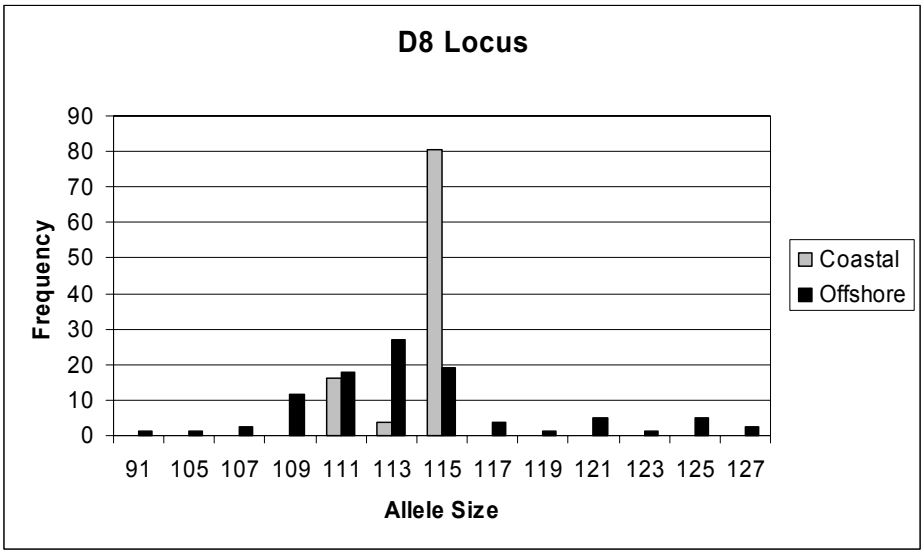
A significant FST value of 0.24 ($p < 0.005$) indicated 24 % of the total variance of allelic frequency distribution was accounted for by differences in variance between the forms: evidence for reduced gene flow between the coastal and offshore dolphins. The software Structure also showed reduced gene flow between the forms with a clear distinction of assignment (**Figure 10**).

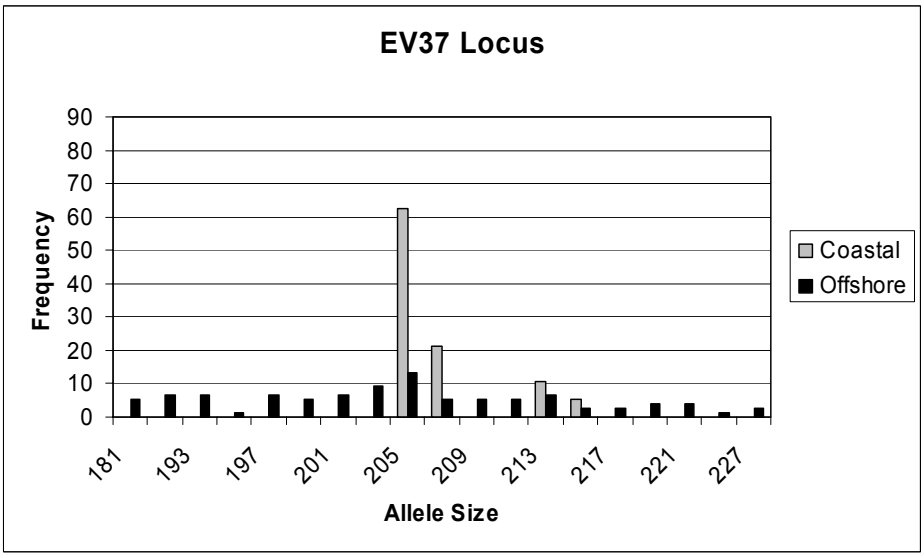
All the loci were within Hardy-Weinberg equilibrium (HWE) for the coastal animals, however, the D8 locus was out of HWE among the offshore dolphins (observed heterozygosity (H_O) = 0.68, expected heterozygosity (H_E) = 0.86, $p < 0.001$). A statistically significant heterozygote deficiency ($p < 0.001$) was identified for this locus among the offshore samples. However, upon excluding the locus from the analysis, the pattern of differentiation did not change

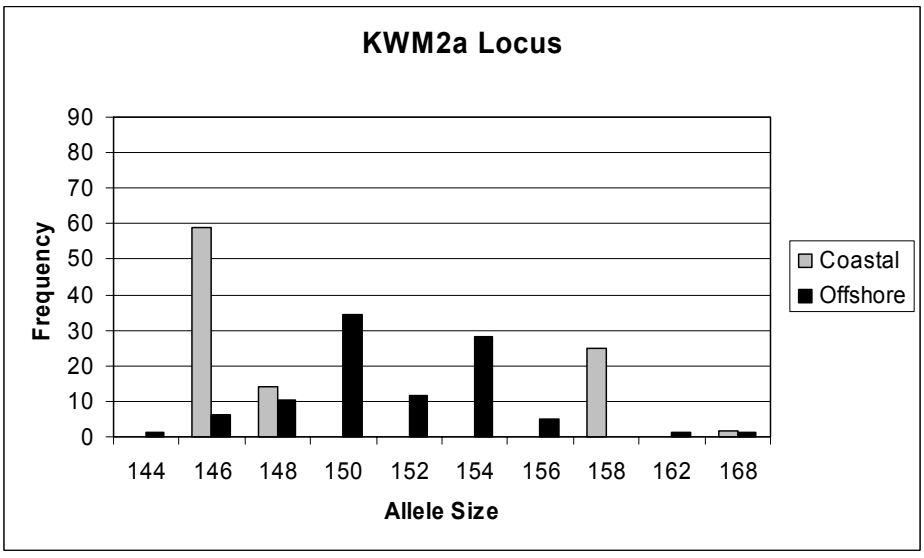
Table 3: The five microsatellite loci genotyped with number of alleles per locus listed for coastal and offshore forms. An asterisk indicates loci with a unique allele found in the coastal form.

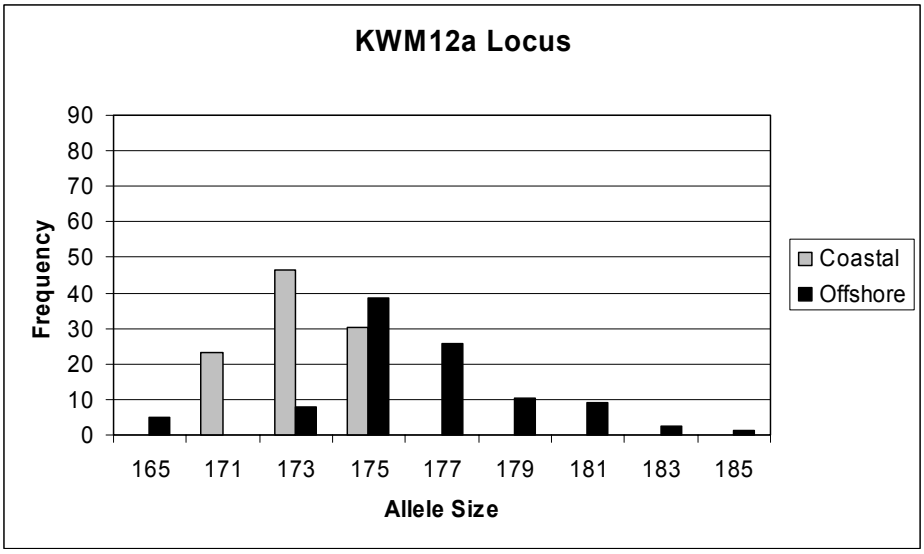
	# of alleles	
Loci	**Offshore**	**Coastal**
D8	13	3
EV37	19	4
KWM2a	9	4 *
KWM12a	8	3 *
TexVet7	7	3

Figure 9: Graphs of allele frequency in the coastal and offshore samples for each microsatellite locus: D8, EV37, KWM2a, KWM12a, and TexVet7.


D8 Locus
Frequency
90
80
70
60
50
40
30
20
10
0
91 105 107 109 111 113 115 117 119 121 123 125 127
Allele Size
Coastal
Offshore


EV37 Locus
Frequency
90
80
70
60
50
40
30
20
10
0
181 193 197 201 205 209 213 217 221 227
Allele Size
Coastal
Offshore


KWM2a Locus
Frequency
90
80
70
60
50
40
30
20
10
0
144 146 148 150 152 154 156 158 162 168
Allele Size
Coastal
Offshore


KWM12a Locus
Frequency
90
80
70
60
50
40
30
20
10
0
165
171
173
175
177
179
181
183
185
Allele Size
Coastal
Offshore

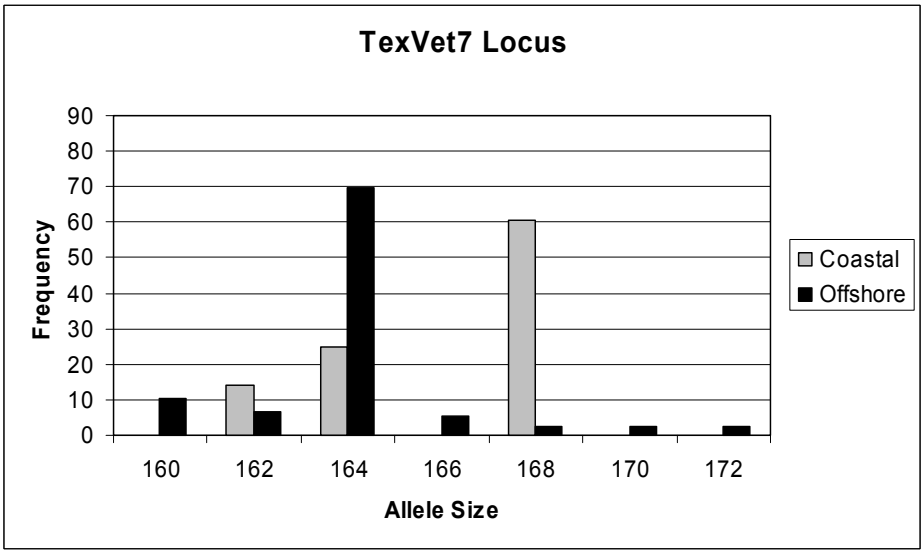

TexVet7 Locus
Frequency
90
80
70
60
50
40
30
20
10
0
160
162
164
166
168
170
172
Allele Size
Coastal
Offshore

Figure 10: Bar plot from Structure software depicting genetic distinctness between coastal and offshore forms from the ENP along the western U.S. Probability is shown on the y axis and each column on the x axis represents an individual. Dark gray represents offshore animals and light gray represents coastal animals.


1.00
0.80
0.60
0.40
0.20
0.00

and thus the locus was included in all analyses. Heterozygote excess was found at the TexVet7 locus in the coastal samples at a significant level ($p = 0.045$). Departure from HWE is not uncommon for microsatellite loci (Smith-Goodwin 1997). The heterozygote deficiency may be a result of the presence of null alleles or the effects of population subdivision (Smith-Goodwin 1997). Linkage disequilibrium was not detected between any pair of loci in either coastal or offshore samples.

Offshore subdivision

In stratifying the offshore samples into Off-SCB and Off-SD, there were fourteen haplotypes identified in each group with three shared haplotypes between them (**Table 2**). An AMOVA for the Off-SCB, Off-SD, and coastal samples resulted in a significant Φ_{ST} value of 0.25 ($p < 0.005$). By pairwise comparison, although a significant Φ_{ST} value was found between the Off-SCB and Off-SD samples, the variation was marginal at 0.06 ($p = 0.020$; **Table 4**). The significant differentiation indicated structure among the offshore bottlenose dolphins, though at a much lower level than between the coastal and offshore forms. A significant difference in variation of haplotypes between the offshore and coastal dolphins ($p < 0.005$) was detected.

The χ^2-test indicated some degree of differentiation of haplotypic frequencies among the Off-SCB and Off-SD samples, but not significant at $p = 0.05$ (**Table 4**). Yet, as expected with no shared haplotypes, samples from Off-SCB and Off-SD had haplotypic frequencies significantly different from the coastal samples ($p < 0.005$).

Table 4: A pairwise comparison of samples from each group for Φ_{ST} and χ^2 values. The Φ_{ST} values are above the diagonal and χ^2 values are below the diagonal. N is the number of individuals sampled from each group. Statistical significance is reported as follows: *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$. For χ^2, Monte Carlo p-values were used.

	N	Off-SCB	Off-SD	Coastal	ETP	Gulf-CA
Off-SCB	**22**	**-**	**0.06***	**0.32*****	**0.08***	**0.11***
Off-SD	**18**	29.0	**-**	**0.37*****	0.06	**0.09***
Coastal	**28**	**50.0****	**46.0****	**-**	**0.43*****	**0.45*****
ETP	**10**	**32.0***	**25.8***	**38.0****	**-**	**0.22***
Gulf-CA	**7**	16.9	21.7	**35.0****	**17.0***	**-**

The F_{ST} value (0.19) for the Off-SCB, Off-SD, and coastal samples was significant ($p < 0.005$). A pairwise comparison of the samples indicated that the Off-SCB and Off-SD samples were not significantly different from one another ($F_{ST} = 0.002$, $p = 0.37$; **Table 5**). The coastal samples were significantly different from the offshore samples, both Off-SCB and Off-SD (Off-SCB: $F_{ST} = 0.25$, $p < 0.005$; Off-SD: $F_{ST} = 0.26$, $p < 0.005$). The Off-SCB and Off-SD samples had similar microsatellite genetic diversity and were both greater compared to the diversity of the coastal samples (**Table 6**).

The D8 locus was out of HWE for the samples from the Off-SCB ($H_O = 0.65$, $H_E = 0.85$, $p = 0.002$) and samples from the Off-SD ($H_O = 0.61$, $H_E = 0.81$, $p = 0.036$). In excluding this locus from the analysis, the F_{ST} value remained significant and thus the locus was included in the analyses.

Genetic diversity in eastern North Pacific *T. truncatus*

To assess genetic variation and phylogeographic concordance within the ENP region, the mitochondrial DNA haplotypes of coastal and offshore animals from along the western United States were compared to a small number of individuals from the ETP and Gulf of California (Gulf-CA) regions. Of the ten ETP bottlenose dolphin biopsy samples analyzed, there were eight haplotypes, one of which was shared with the Off-SD animals. All but one of the haplotypes (haplotype # 31) from the ETP was represented by a single individual. Only four haplotypes were identified among the seven individuals from the Gulf-CA, all of which were shared with the offshore samples (three with Off-SCB and one with

Table 5: A pairwise comparison of coastal and offshore samples for F_{ST} values. N is the number of individuals sampled from each group. Statistical significance is reported as follows: *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$.

	N	Off-SCB	Off-SD
Off-SCB	**20**	**-**	
Off-SD	**18**	0.002	**-**
Coastal	**28**	**0.25*** **	**0.26*** **

Table 6: Gene diversity for mtDNA and microsatellite DNA for each group of samples.

	mtDNA	msats
Off-SCB	**0.95 ± 0.03**	**0.77 ± 0.44**
Off-SD	**0.97 ± 0.03**	**0.72 ± 0.43**
Coastal	**0.78 ± 0.04**	**0.54 ± 0.32**
ETP	**0.93 ± 0.08**	**---**
Gulf-CA	**0.71 ± 0.18**	**---**

Off-SD). Including the ETP and Gulf-CA samples with the coastal and offshore samples, there were a total of 37 haplotypes with 40 variable sites (**Table 2**).

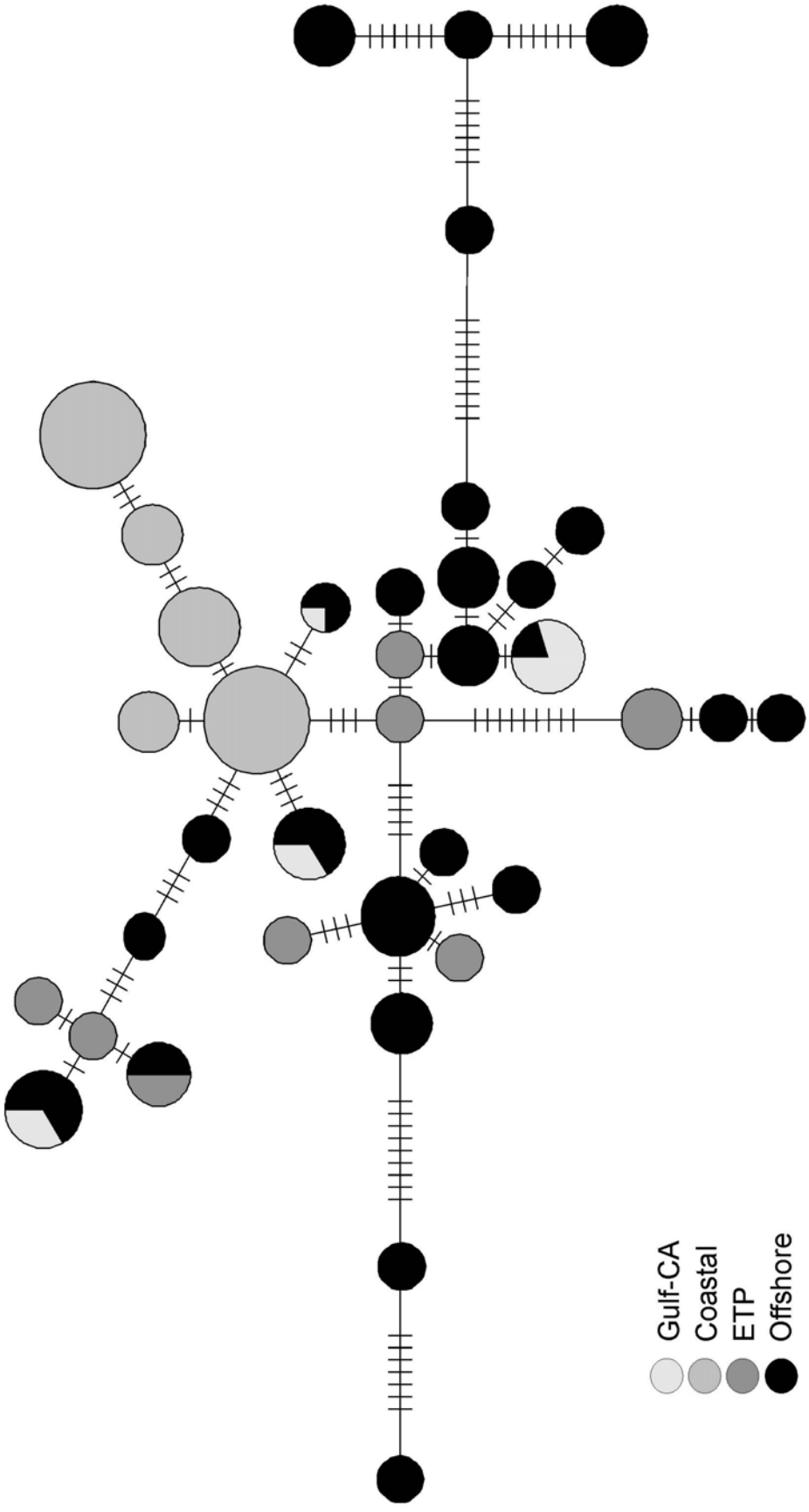
After incorporating the ETP and Gulf-CA haplotypes into the minimum spanning network, the coastal haplotype (# 27) and the offshore haplotype (# 8) had the greatest number of branches stemming from them (six branches, **Figure 11**). Haplotype # 8 and haplotype #27 had the highest frequency within the offshore and coastal samples, respectively. The coastal samples retained exclusive branches with closely related haplotypes. Haplotypes from the ETP and Gulf-CA were distributed throughout the network among the offshore (combined Off-SCB and Off-SD) haplotypes, but with no clear grouping or distinction, thus indicating no apparent phylogeographic concordance in the ENP region.

For mtDNA, the offshore and ETP samples had a greater diversity than the coastal samples (**Table 6**). The Gulf-CA samples had the lowest diversity for mtDNA, however, there were only seven samples sequenced from that region, four of which had the same haplotype. Therefore, it may not be a true representation of gene diversity among the Gulf-CA dolphins (see section "Inclusion of Gulf of California coastal and offshore sequences").

Population subdivision for eastern North Pacific *T. truncatus*

Samples from Off-SCB, Off-SD, Coastal, ETP, and Gulf-CA had a significant Φ_{ST} value of 0.23 ($p < 0.005$), suggesting population structure within the ENP region. The coastal samples had significant differentiation ($p < 0.005$) in variation from samples of the Off-SCB, Off-SD, ETP, and Gulf-CA with Φ_{ST} values ranging from 0.32 to 0.45 (**Table 4**). The greatest difference was between

Figure 11: Minimum spanning network of haplotypes for coastal, offshore, ETP, and Gulf-CA samples. Each circle represents a haplotype and the size of the circle is relative to the number of individuals sequenced with that haplotype. Pie chart symbols represent shared haplotypes between samples of different groups. The ticks on each branch represent number of base pair differences between haplotypes.


Gulf-CA
Coastal
ETP
Offshore

the coastal samples and Gulf-CA samples. Although, the Gulf-CA samples were significantly different from the ETP, Off-SCB, and Off-SD samples, the Φ_{ST} values were lower than the differentiation found between the coastal and offshore forms. This suggests the coastal dolphins have had reduced gene flow from the dolphins of the other regions for a longer period of time than have the Gulf-CA dolphins.

Of the Off-SCB, Off-SD, and ETP samples, the ETP samples had the greatest differentiation ($\Phi_{ST} = 0.22$, $p < 0.05$) from the Gulf-CA samples while the Off-SD samples had the least ($\Phi_{ST} = 0.09$, $p < 0.05$). This suggests more recent gene flow between the Off-SD dolphins and the Gulf-CA dolphins than their closer geographic neighbors, the coastal and ETP dolphins.

The χ^2-test revealed the coastal dolphins to be significantly different ($p < 0.005$) in haplotypic frequency from the dolphins of the Off-SCB, Off-SD, ETP, and Gulf-CA (**Table 4**). This was expected as the coastal samples had no shared haplotypes with any of the other samples. The ETP dolphins also had significant differences ($p < 0.05$) in haplotypic frequencies from the Off-SCB, Off-SD, and Gulf-CA dolphins. As indicated on the haplotype frequency table, there was only one haplotype from the ETP samples that was shared with another sample group, the Off-SD samples. However, only ten samples were sequenced from the ETP which has an estimated population size of over 200,000 animals (Wade and Gerrodette 1993) and thus may not be a true representation of dolphins from that area.

Inclusion of Gulf of California coastal and offshore sequences

Incorporating Segura García's (2004) Gulf of California coastal and offshore bottlenose dolphin mtDNA sequences with the data of the current study, the genetic differentiation trends as presented above do not change except for a decrease in differentiation between the coastal population and Gulf-CA dolphins, though still remaining significant ($p < 0.005$). Segura García (2004) identified 28 haplotypes among eighty-six coastal and offshore dolphins of the Gulf of California. Ten of those haplotypes matched haplotypes of the current study from Off-SCB, Off-SD, and the ETP samples (**Table 7**). No haplotypes were shared with the ENP coastal population of the current study. There were two indels, only one of which was found in the Gulf of California sequences. The indel at site 129 was in a Gulf-CA offshore sample and shared with Off-SCB samples.

With the addition of sequences from the Gulf of California, the minimum spanning network continued to indicate no apparent phylogeographic concordance for the ENP region (**Figure 12**). The Gulf-CA coastal and Gulf-CA offshore haplotypes were distributed throughout the network with no clear grouping or distinction. The ENP coastal samples maintained their exclusive branches, however, one Gulf-CA coastal haplotype (TTGC31) and one Gulf-CA offshore haplotype (TTGC29) branched off a high frequency ENP coastal haplotype (#29) by one and eight nucleotide differences, respectively. Haplotype TTGC31 was identified for five coastal individuals in the northern part of the Gulf and haplotype TTGC29 was identified for one offshore individual in the southern part of the Gulf.

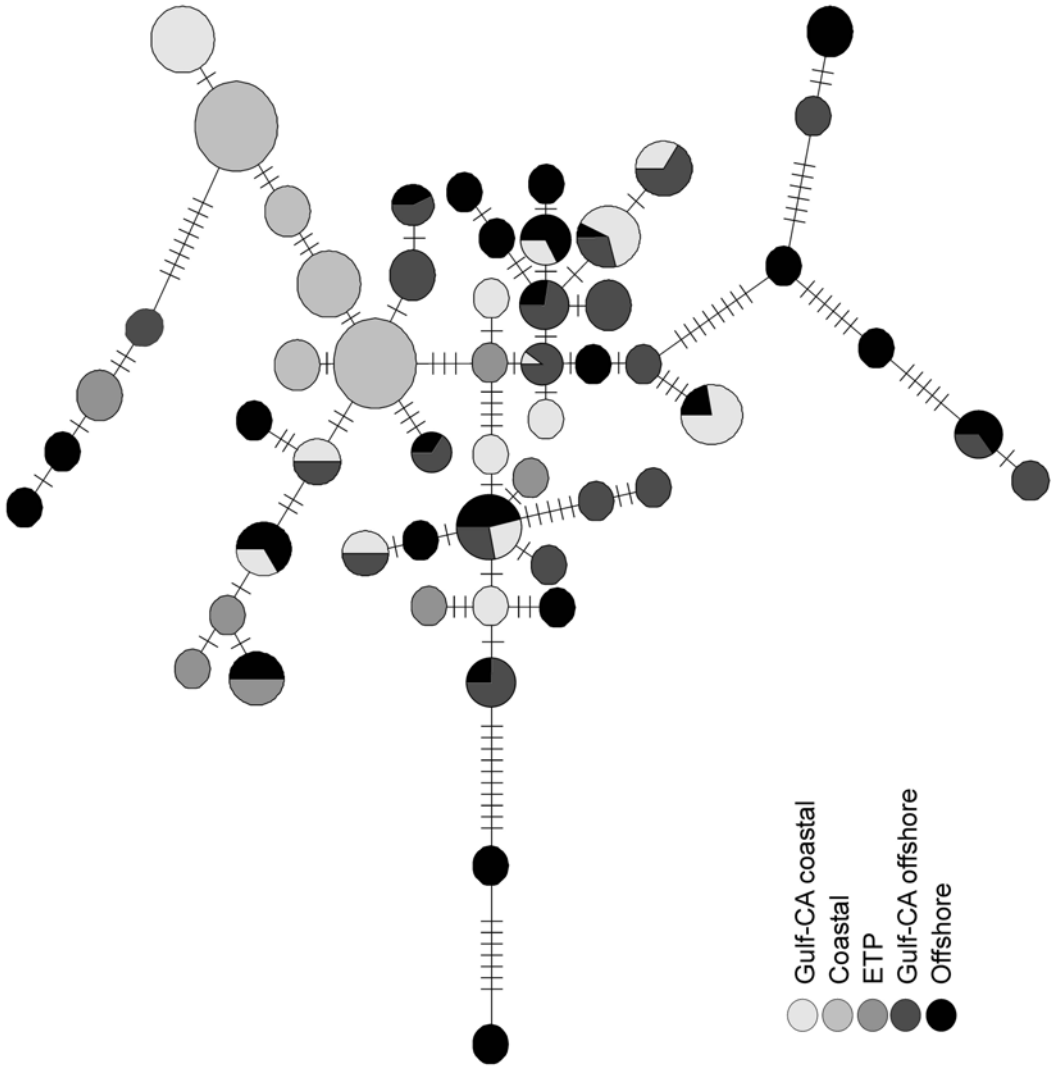
Table 7: Polymorphic sites and haplotype frequencies of the 402 base pairs sequenced within the mtDNA control region for samples from each of the five areas, including coastal and offshore forms from the Gulf-Ca (Segura García 2004). In addition to the samples sequenced from Off-SCB, Off-SD, Coastal, and ETP, sequences from Segura García (2004) of coastal and offshore *T. truncatus* in the Gulf of California were added to Gulf-CA samples of this study and designated as Gulf-CA coastal and Gulf-CA offshore. Haplotypes from Segura García (2004) are labeled as TTGC##. Number of individuals from the current study (JL) and number of individuals from the Segura García (2004) study (IS) are indicated in the Gulf of California columns as (JL/IS) respectively.

	Polymorphic Sites					Haplotype Frequencies					
	11111	1111222222	2222222222	2233333333	3333	Off-SCB	Off-SD	Coastal	ETP	Gulf-CA coastal	Gulf-CA offshore
	022399900024	5578014466	7777778888	9900025779	9999					(JL/IS)	(JL/IS)
	703227916999	0112565859	2457890237	3423597030	1234	N=22	N=18	N=29	N=10	N=35	N=53
1;TTGC07	-GATATTTAT-C	ATGTCACGCC	ACATTTTCCA	TCCATTCCTA	TCTC	1				4/6	-/4
2;TTGC15	-.....C...-.	....	......C...	C....C.T..	....	2				1/5	
3;TTGC26	-..CG.C..CTT	......TA..	.....C....	.....C.T..	....	2					-/1
4	-.....C...-.	....T...T.	.......T..	.T...C.T.G	C...	1					
5	-..C.CC..C-T	....T.T..T	....CC.T..	...G.C.T..	....	1	1				
6;TTGC32	-.....C...-.	....T.T.T.	.......T..	.....C...G	C...	2					-/6
7;TTGC23	-.....CC..-.	....	....	C....C....	....	3					1/3
8;TTGC02	-.....C...-.	....T...T.	.......T..	.T...C...G	C...	4	3			-/4	-/4
9	-A...CC.G.-T	.C......A.	G....C.T..	...G.C....	....	1					
10	-.........-.	....	....	.T.....T..	C...		1				
11	-........C-.	.........T	..G.......	.T........	....		1				
12	A.....C...-.	........T.	.......TT.	CT.G.C..C.	....		1		1		
13;TTGC08	-.........-.	....	....	.T........	C...		2			-/2	
14;TTGC11	-.........-.	....	....	.T........	....	1	1				-/5
15	A....CC...-T	....	.TG.CC....	...G.C....	C...		1				
16	-.....C...-.	........T.	.........G	CT.G.C..C.	....		1				
17;TTGC03	-.....C...-.	........T.	....	CT...CT.C.	....		1				-/2
18	-.........-.	....	....	.T...C.T..	....		1				
19	A.....C...-.	........T.	.......T..	CT.G.C....	....		2			1/-	
20	-..C..C..C-T	....T.....	....CC....	.TT..C.T..	C...	1					
21	A.....C...-T	......T...	.A..CC.T..	.....C....	C..T	1					
22	-..C..C..C-T	G.....T...	....CC....	.....C.T..	....	1					

	Polymorphic Sites					Haplotype Frequencies					
	11111	1111222222	2222222222	2233333333	3333	Off-SCB	Off-SD	Coastal	ETP	Gulf-CA coastal (JL/IS)	Gulf-CA offshore (JL/IS)
	022399900024	5578014466	7777778888	9900025779	9999						
	703227916999	0112565859	2457890237	3423597030	1234	N=22	N=18	N=29	N=10	N=35	N=53
23	-A...CC.G.-T	.C......A.	.....C.T..	...G.C....	....	1					
24	-.........C-.	....	..G.......	.T........	....		1				
25	-.....C...-.	....T...T.	.......T..	...G.....G	C...		1				
26	-.....C...-.	........TT	....	C..G.C.T..	....			3			
27	-.....C...-.	.........T.	....	C....C....	....			9			
28	-.....C...-.	........TT	....	C....C....	....			6			
29	-.....C...-.	.........T.	........T.	C..G.C.T..	....			9			
30	-.....C...-.	.........T.	....	C...CC....	....			2			
31	-A...CC.G.-T	........A.	.....C.T..	...G.C....	....				3		
32	A.G...C...-.	.........T.	.......T..	CT.G.C..C.	....				1		
33	-.....C...-.	....	....	.T...C....	....				1		
34	-.....C.G.-.	....T...T.	.......T..	.T...C...G	C...				1		
35;TTGC13	-..........-.	....	....	.T...C....	....				1		-/7
36	A.....C...-.	.........T.	.......T..	CT.G.C..C.	....				1		
37	-.....C...-.	...CT...T.	...C...T..	.....C...G	C...				1		
TTGC01	-....CC...-.	....T...T.	.......T..	.T...C.T.G	C...					-/1	-/1
TTGC04	-.....C...-.	.........T.	....	CT.G.C....	....					-/1	-/1
TTGC06	-.....CC..-.	.........T.	....	C....C....	....						-/4
TTGC09	-.....C...-.	....T...T.	.......T..	.....C...G	C...					-/1	
TTGC10	-..........-.	....	....	....	C...					-/1	-/2
TTGC12	-..........-.	....	....	CT........	....						-/4
TTGC14	-.....C...-.	....	...C......	.T...C....	....					-/1	
TTGC17	-.....C...-.	....TG..T.	.......TT.	CT...C...G	CTC.						-/1
TTGC18	-.....C...-.	....T...T.	.......T..	.T...C....	C...					-/1	

	Polymorphic Sites					Haplotype Frequencies					
	11111	1111222222	2222222222	2233333333	3333	Off-SCB	Off-SD	Coastal	ETP	Gulf-CA coastal	Gulf-CA offshore
	022399900024	5578014466	7777778888	9900025779	9999					(JL/IS)	(JL/IS)
	703227916999	0112565859	2457890237	3423597030	1234	N=22	N=18	N=29	N=10	N=35	N=53
TTGC22	-.....C...-.	....	....	.T...C.T..	....						-/1
TTGC24	-....CC...-.	....T...T.	.......T..	.T...C...G	C...						-/2
TTGC25	-.....C...-.	....T.T.T.	.......TT.	CT...C...G	CTC.						-/1
TTGC27	-..CG.C..CTT	..A...TA..	.....C....	.....C.T..	....						-/1
TTGC28	-..C.CC..C-T	....T....T	....CC.T..	.....C.T..	....						-/1
TTGC29	-A...CC.G.-T	........A.	.....C.TT.	...G.C.T..	....						-/1
TTGC30	-.........-.	....	........T.	.T...C....	....					-/1	
TTGC31	-.....C...-.	........T.	........T.	CT.G.C.T..	....					-/5	

Figure 12: Minimum spanning network of haplotypes from Gulf-CA coastal, Gulf-CA offshore, Coastal, Offshore (Off-SCB + Off-SD), and ETP samples. Each circle represents a haplotype and the size of the circle is relative to the number of individuals sequenced with that haplotype. Pie chart symbols represent shared haplotypes between samples of different groups. The tick marks on each branch represent number of base pair differences between haplotypes.


Gulf-CA coastal
Coastal
ETP
Gulf-CA offshore
Offshore

The minimum spanning network is not absolute but just one representation of how haplotypes are related. According to the haplotype distance matrix that is used to construct the network, the next most closely related haplotype to TTGC31 was TTGC04, which was identified for one coastal and one offshore sample in the central and southern portions of the Gulf. Haplotype TTGC04 branched from the other high frequency ENP coastal haplotype (#27) by two nucleotide differences. The next most closely related haplotype, each ten base pairs in distance, to haplotype TTGC29 was TTGC28, TTGC24, haplotype #5, and haplotype #26. Haplotypes TTGC28 and TTGC24 were composed of Gulf-CA offshore samples, while haplotype #5 was of ENP offshore samples, and haplotype #26 was of ENP coastal samples.

With the addition of the sequences from Segura García (2004) to the seven samples of the current study, the mtDNA gene diversity increased for the Gulf of California dolphins from 0.71 ± 0.18 to 0.95 ± 0.01. When divided into coastal and offshore forms, the Gulf-CA coastal animals had a lower diversity of 0.86 ± 0.04 than the Gulf-CA offshore animals with a diversity of 0.94 ± 0.01. The ENP coastal animals continued to have the lowest mtDNA diversity at 0.78 ± 0.04.

Population subdivision

The added sequences from the Gulf of California resulted in a decrease of Φ_{ST} among the samples from the ENP region (Off-SCB, Off-SD, Coastal, ETP, Gulf-CA coastal, and Gulf-CA offshore). The value remained significant ($p < 0.005$) but decreased from 0.23 to 0.15. Since Φ_{ST} is calculated by genetic distance and haplotype frequency, this decrease is most likely due to the sharing

of Gulf-CA haplotypes with ENP offshore samples and the increase in number of haplotypes within the Gulf-CA sample set.

The ENP coastal samples maintained a significant differentiation ($p < 0.005$) of variation from samples of the Gulf-CA (both coastal and offshore) (**Table 8**). This differentiation was greater than the Φ_{ST} values calculated between the ENP offshore or ETP samples and Gulf-CA animals. This distinct differentiation of the ENP coastal animals suggest these animals have had reduced gene flow from dolphins of the other ENP areas for some time. Walker (1981) originally proposed the Gulf of California coastal and offshore dolphins to be synonymous with the ENP coastal and ETP offshore animals, however, the data presented here disagree with that claim. The AMOVA results indicate significant differentiation between the ENP coastal and Gulf-CA coastal, as well as between the ETP and Gulf-CA offshore samples.

There was greater differentiation between the Gulf-CA samples (coastal and offshore) to the ENP coastal samples than to the ENP offshore samples (Off-SCB and Off-SD). This differentiation suggests there has been more recent gene flow between the ENP offshore animals and the Gulf-CA dolphins than the ENP coastal animals have had with the Gulf-CA dolphins.

Samples from Off-SD were not significantly different from the ETP, Gulf-CA coastal, or Gulf-CA offshore samples, whereas samples from Off-SCB were. This suggests recent or ongoing gene flow between Off-SD and ETP and Gulf-CA dolphins and supports subdivision among the ENP offshore animals. Significant genetic differentiation ($p = 0.09$) was not found between the Gulf-CA coastal and

Table 8: A pairwise comparison of samples from each group for Φ_{ST} and χ^2 values including Gulf of California samples from Segura García (2004). The Φ_{ST} values are above the diagonal and χ^2 values are below the diagonal. N is the number of individuals sampled from each group. Statistical significance is reported as follows: *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$. For χ^2 test, Monte Carlo p-values were used.

	N	Off-SCB	Off-SD	Coastal	ETP	Gulf-CA Coastal	Gulf-CA Offshore
Off-SCB	**22**	**-**	**0.06***	**0.32*****	**0.08***	**0.10****	**0.06***
Off-SD	**18**	29.0	**-**	**0.37*****	0.06	0.04	0.03
Coastal	**28**	**50.0****	**46.0****	**-**	**0.43*****	**0.29*****	**0.30*****
ETP	**10**	**32.0***	**25.8***	**38.0****	**-**	**0.18****	**0.14***
Gulf-CA Coastal	**35**	**38.4****	**37.9***	**63.0****	**45.0****	**-**	0.02
Gulf-CA Offshore	**53**	**38.8***	**54.0****	**81.0****	**56.4****	**60.8****	**-**

Gulf-CA offshore, suggesting there is current or recent gene flow between the two ecotypes in this region.

The Gulf of California dolphins had a significant difference ($p < 0.05$) in haplotypic frequency from dolphins of the Off-SCB, Off-SD, Coastal, and ETP. As expected with no shared haplotypes between them, the χ^2 values were largest between the ENP coastal dolphins and Gulf-CA dolphins, both coastal and offshore. Although the Gulf-CA dolphins shared ten haplotypes with samples from other ENP groups, the majority of haplotypes were unique to the Gulf region, thus providing large χ^2 values.

CHAPTER 5: DISCUSSION

Both mitochondrial and nuclear markers indicated that coastal and offshore bottlenose dolphins in the eastern North Pacific Ocean, along the western United States, are genetically distinct populations. There were no shared haplotypes between the two ecotypes. The genetic variation and allelic richness were substantially less in the coastal population than in the offshore population.

It is hypothesized that the coastal population is a result of a founder effect by the offshore population and subsequently has remained a small population for some time, in order to reach nucleotide fixation. The low genetic diversity of five haplotypes, with exclusive branching and small genetic distance, found among the 29 coastal animals support this hypothesis. Abundance estimates for the coastal population have also reported it to be a small population for the past twenty years (Hansen 1990; Defran and Weller 1999; Carretta et al. 2005; Dudzik et al. in press).

The minimum spanning network of the coastal and offshore populations depicted the coastal animals as the founding population since the haplotype with the most branches stemming from it within the network was of the coastal population. A haplotype with a high frequency, central position (such as the common coastal haplotype # 27), and connection to many population-specific haplotypes, can indicate the expansion of discrete populations from an ancestral population (Lavery at el. 1996). From the ancestral haplotype, population-specific haplotypes can evolve through mutation, limited gene flow, or drift (O'Corry-Crowe et al. 1997).

The suggestion that the coastal dolphins are the founder population may simply be due to the small sample size of this study. Upon increased sampling, the central coastal haplotype could be revealed within the offshore population. Since approximately ten percent of the coastal population was sampled and only an estimated 1.3 % of the offshore population, it is more likely with further sampling that a coastal haplotype will be found within the offshore population than an offshore haplotype found in the coastal population.

The inclusion of ETP and Gulf-CA haplotypes into the minimum spanning network resulted in the two highest frequency coastal and offshore haplotypes to have the most branches stemming from them. Having increased branching from an offshore haplotype with the addition of samples suggests that with further sampling, the network may indicate that the offshore dolphins are the founder population. A minimum spanning network is not absolute, but one representation of genetic distance between haplotypes. According to the distance matrix upon which the minimum spanning network is based, the Gulf-CA coastal haplotype (TTGC31) that branched off the end of an exclusive ENP coastal branch could also have been drawn branching off a Gulf-CA coastal haplotype outside of the exclusive ENP coastal haplotypes. Similarly, the Gulf-CA offshore haplotype (TTGC29) could also have been drawn elsewhere in the network branching off other offshore Gulf-CA or ENP offshore haplotypes rather than branching from an ENP coastal haplotype. Yet, since the minimum spanning network depicted these Gulf-CA haplotypes as branching off an ENP coastal haplotype, it suggests there may have been gene flow between the ENP and Gulf-CA coastal animals at

one time, or there may have been a common ancestor haplotype to both populations.

The distribution of haplotypes within the network suggests there is no apparent phylogeographic concordance observed for the ENP region. With increased sampling and a more extensive genetic study of ETP bottlenose dolphins, perhaps a phylogeographic structure of some level would be apparent.

Pairwise differences were largest between the coastal population and the Off-SCB, Off-SD, ETP, Gulf-CA coastal, and Gulf-CA offshore populations for Φ_{ST} and χ^2 values. This significant differentiation suggests reduced gene flow for some time between the coastal population and the other ENP populations. Due to the small number of samples from the ETP used in this study, it is difficult to determine the extent of genetic differentiation in the eastern North Pacific region, outside of the coastal and offshore forms along the western U.S. However, since differentiation was found using such a small sample size, there is some evidence for population structure in this region.

The structure indicated by the analysis of seven samples from the Gulf-CA was maintained with the addition of eighty-six sequences from Segura García (2004). With an estimated abundance of 35,000 dolphins in the Gulf of California, the increased sample set represented 0.2 % of the Gulf bottlenose dolphins. The increased sample size and designation into coastal and offshore forms of the Gulf-CA samples supported significant genetic differentiation between the ENP coastal population and the Gulf-CA dolphins. These data refute Walker's (1981) proposal that the coastal bottlenose dolphins along southern

California were synonymous with the coastal dolphins within the Gulf of California.

The data suggested structure among the offshore dolphins of the ENP, within the Southern California Bight. This structure was supported by three findings in the results: 1) a significant Φ_{ST} value between the Off-SCB and Off-SD samples 2) the Off-SCB samples having significant differentiation from the other four ENP populations 3) the Off-SD samples not having significant differentiation from the ETP, Gulf-CA coastal, and Gulf-CA offshore samples. Although there was not a significant F_{ST} value found between the two offshore groups, this finding is not surprising given the power of the nuclear marker and a small sample size. It is common in marine mammal species to find a greater pattern of structure in mitochondrial markers, due to their lower effective population size, than in nuclear markers (Hoelzel et al. 2002).

Given the large size of the offshore population, the AMOVA analysis (Φ_{ST} value) may have falsely indicated structure within the population due to high mtDNA gene diversity and the presence of many unique haplotypes in both Off-SCB and Off-SD strata. Although, many of the haplotypes in these strata were represented by one or two individuals, the most common haplotype (#8) was found at a high frequency in both offshore groups. This common shared haplotype could indicate that these two strata are derived from a single population with many low frequency haplotypes. A haplotypic distribution such as this could lead to the erroneous detection of significant structure between strata composed of small sample sizes. On the other hand, the results of this study could accurately

indicate subdivision among the offshore animals in this region. Extensive sampling is required to accurately assess the structure of this potentially highly diverse population.

There was sampling bias of offshore animals in this study. Although NOAA research cruises of the last thirty years have established a relatively uniform distribution of effort along the west coast of the United States, biopsy samples collected, comprising the Off-SCB group, were restricted to summer and fall months of designated years. Samples of the Off-SD group were collected opportunistically by small boat sampling effort on six outings between 2000 and 2004 in summer and fall months. The eighteen samples collected were composed of six *T. truncatus* groups all within twenty kilometers from the San Diego county shore. Thus, the offshore samples were collected in bias to sampling years, seasons, and locations, and may not represent random sampling of the population.

Though the frequency of haplotypes between the ENP offshore (both Off-SCB and Off-SD) and ETP populations was significantly different, as indicated by χ^2 analysis, the AMOVA test indicated significant differentiation, though marginal, only between Off-SCB and ETP populations. This low level differentiation suggests that though there are haplotypic frequency differences between the populations, there may be recent or ongoing gene flow between them. The Φ_{ST} data suggest more recent gene flow between the Off-SD and ETP samples than between the Off-SCB and ETP samples, a finding that supports population structure within the offshore animals of the ENP region.

Both Off-SD and Off-SCB strata had lower genetic differentiation from the Gulf-CA populations, than from their closest geographic neighbor, the ENP coastal population. Even though the Gulf-CA populations are located a great distance from the ENP offshore dolphins, there seems to be more recent or ongoing gene flow between them than between the ENP offshore and coastal populations, whose ranges are as close as five kilometers from each other. This coastal vs. offshore genetic distinctness is curious given the close proximity and lack of geographic barriers separating the two ecotypes.

The population structure detected between the coastal and offshore forms in the ENP could be attributed to behavioral specialization for local resources, social structure, or historical environmental change (Hoelzel 1998). Although focused ecological studies have not been conducted on the offshore animals, Defran et al. (1999) documented the coastal animals to move freely up and down the coast within their narrow 'coastal corridor' in relation to prey availability. Walker (1981) showed the two ecotypes to exploit the unique resources of their habitats, as the stomach contents of offshore animals consisted predominantly of epipelagic and mesopelagic fish while the contents of coastal animals were of littoral and sublittoral species.

In a study done by Weller (1991) on the social ecology of the coastal bottlenose dolphins in the ENP, in the waters along San Diego county, it was discovered that contrary to many other animal species which display stable social groups often with permanent members, such as killer whale pods, the coastal dolphins comprise variable size groups that divide and coalesce regularly. This

dynamic and complex affiliation pattern is thought to be an adaptation for foraging efficiency rather than protection from predators (Weller 1991). Defran and Weller (1999) documented coastal group sizes to range from one to 100 individuals, with the most common composed of two to 15 animals. DeDecker et al. (1999) observed offshore dolphins to also have variable size groups ranging from three to 70 individuals, with smaller groups typically associated with Risso's dolphins, *Grampus griseus*.

Environmental change may also have influenced the population structure of coastal and offshore dolphins in the ENP (Hoelzel 1998). A recently important event for the dolphins was the 1982-1983 El Niño that brought an incursion of warm water northward (Wells et al. 1990). This extension of warm water increased primary productivity along north-central California, which in turn shifted the prey distribution northward (Defran et al. 1999). As opportunistic foragers, coastal bottlenose dolphins extended their range from the Los Angeles area as far north as Monterey Bay to follow the availability of their prey (Wells et al. 1990; Weller 1991). The offshore animals also were documented to extend their range during this warm water period into Oregon and Washington waters, though remaining in the pelagic area (Forney and Barlow 1998).

***T. truncatus* study comparisons**

In comparison to other *T. truncatus* genetic studies, the results in this study were in concordance with the global findings of low genetic variation within coastal populations (Dowling and Brown 1993; Curry 1997; Smith-Goodwin 1997; Hoelzel et al. 1998b; Krützen et al. 2004; Natoli et al. 2004;

Segura García 2004; Natoli et al. 2005; Sanino et al. 2005). There were no shared haplotypes found between the coastal and offshore ecotypes of the ENP, WNA, and eastern South Pacific (Curry 1997; Hoelzel et al. 1998b; Natoli et al. 2004; Sanino et al. 2005). In the Gulf of California and South Africa, however, there was overlap of haplotypes between the two forms, suggesting less restricted gene flow between the ecotypes in those regions (Hoelzel et al. 1998b; Segura García 2004; Segura et al. in prep).

Although Curry (1997) found no shared haplotypes among offshore bottlenose dolphins from different ocean basins, the analysis of variance indicated low levels of genetic differentiation among the pelagic dolphins of the Pacific, Atlantic, and Indian Oceans. The analysis showed the greatest differentiation to be between the Pacific offshore samples and the offshore samples of the other two ocean basins. However, the sample size was small and the data was not comparable to the data set of the current study for further analysis. Dowling and Brown (1993) also examined genetic differentiation between bottlenose dolphins of the Atlantic and Pacific Oceans. Their study found distinct haplotypic differences, indicating a lack of gene flow between ocean basins. However, the Pacific Ocean was represented by a small number of samples collected from the Timor Sea, north of Australia, and thus are not likely a true representation of the region.

Although fixed nucleotide substitution differences and monophyly were not found in the *T. truncatus* populations of the ENP as they were in the WNA coastal and offshore populations (Curry 1997; Hoelzel et al. 1998b), the results

between the two regions were similar. In both regions, the offshore population had greater haplotypic diversity (0.88 WNA vs. 0.96 ENP) than the coastal population (0.43 WNA vs. 0.76 ENP). The low variation of coastal haplotypes found in the ENP (five haplotypes for 29 individuals) was similar to the findings in the WNA (five haplotypes for 29 individuals). Curry (1997) found nine haplotypes among the 16 coastal WNA dolphins and six haplotypes among the 42 coastal northern Gulf of Mexico dolphins.

There were no shared haplotypes between WNA coastal and offshore populations and though alleles were shared between the WNA populations, there were significant allelic frequency differences (Hoelzel et al. 1998b). Although F_{ST} values for the coastal and offshore populations of the WNA and ENP were similar, the WNA had a greater Φ_{ST} value of 0.60 (compared to the ENP with a Φ_{ST} value of 0.27). Reduction in gene flow may have been more recent for the ENP coastal and offshore populations, explaining the lack of monophyly and fixed nucleotide differences between the ecotypes.

The greater genetic differentiation found between the WNA coastal and offshore populations may be contributed by key ecological and oceanographic differences between the ENP and WNA, along the coasts of the United States. The WNA is characterized by numerous bays, lagoons, and estuaries, as well as a wide continental shelf (Kenney 1990; Curry 1997). This environment supports many resident coastal bottlenose dolphin populations as well as seasonal migrants (Kenney 1990; Scott 1990). The wide continental shelf encourages further offshore swimming by coastal animals, resulting in the two ecotypes being

sympatric in some regions (Torres et al. 2003). Yet, even with this overlap of range, there is strong differentiation between the forms. The philopatry observed among WNA coastal animals can cause lower genetic diversity within populations, as is often observed in isolated, small resident groups.

The ENP has fewer bays and lagoons and a narrow continental shelf (Hansen 1990). The coastal animals of this region do not show site fidelity or seasonality but have a large 'home range' extending along the coast of California and are a panmictic population (Defran et al. 1999). The two forms along this coast are parapatric populations, where bottlenose dolphins have not been observed between the coastal and offshore ranges, a band of approximately 1 to 4 km from the shore (Defran and Weller 1999).

Another key difference between the ENP and WNA regions of the United States is population size. The WNA, along the eastern coast of the U.S., has a greater abundance of bottlenose dolphins than the ENP, along the western U.S. There are five established stocks of coastal animals in the U.S. EEZ of the WNA, comprised of approximately 15,000 animals overall (NOAA 2005). The smallest stock, off northern Florida, is 450 individuals, while the largest stock is 10,600 individuals off central Florida (NOAA 2005). The offshore population in the WNA region is estimated at 71,400 animals (NOAA 2005). As previously mentioned, the coastal population of the ENP, off California is estimated at 323 animals and the offshore population at 3,000 individuals (Carretta et al. 2005; Dudzik et al. in press). However, there is a large population of bottlenose

dolphins in the ETP, estimated at over 200,000 animals (Wade and Gerrodette 1993).

Management

The U.S. Marine Mammal Protection Act of 1972 (MMPA) and the Endangered Species Act of 1973 (ESA) direct management efforts on populations below the species level. These two acts seek to maintain a species, as well as distinct evolutionarily unique populations of a species, from extinction (Dizon 2002). In order to successfully conserve and manage a cetacean species, it is important to determine population boundaries and the extent of gene flow across those boundaries (Dizon 2002). If significant gene flow exists between populations, a loss in one population could potentially be replenished by an adjacent population. However, in the absence of gene flow or with reduced gene flow between populations, an adjacent population would not be likely to replenish the loss within another population.

The genetic distinctness found between coastal and offshore bottlenose dolphins in the ENP, off the western U.S., supports the present management designation of separate coastal and offshore stocks and highlights the importance of continued monitoring of the ENP coastal population. This population is small in number (300-500 individuals) and inhabits a narrow coastal home range ($\leq$ 1 km from shore) (Defran and Weller 1999, Defran et al. 1999). Therefore, it is particularly susceptible to a variety of human related threats, including: incidental bycatch in fishing operations, habitat alteration, vessel traffic, underwater noise, and pollution (Wilson et al. 2000; Dudzik et al. in prep). It is believed that

pollution may account for viral outbreaks in marine mammals which can often result in unusual mortality events (i.e. die-off) (Hall et al. 1992).

A large die-off of coastal animals would not only result in the loss of genetic variation but could also result in the loss of local adaptation and culturally transmitted population traits. The loss of genetic variation could reduce the survival and fitness of individuals while the loss of culture could result in the extinction of novel shared behaviors created or maintained by social learning within the population (Norris 2002). Although cultural differences among ENP dolphins have not been studied, there is evidence that cultural traits related to development of local adaptations can arise in bottlenose dolphins as a species. For example, dolphins in Shark Bay, Australia have been observed using marine sponges as a foraging tool, a behavior which seems to largely be passed between mothers and their daughters (Krützen et al. 2005).

In addition to behaviors being learned between generations, behaviors can also be learned among individuals within a generation, perhaps to adapt to an environmental change or anthropogenic threat (Whitehead et al. 2004). Given that the coastal population of the ENP, along the west coast of the U.S., has maintained a relatively stable population size for the last twenty years or more (Dudzik et al. in prep), it is possible that they have developed specific cultural behaviors to deal with the unique challenges of living in their habitat. Therefore, it is important to continue monitoring the health and habitat of the seemingly vulnerable coastal dolphins as any event causing mortality above natural rates

could have long-lasting reproductive, survival, and demographic effects on this small population.

Future Work

As this study provided the first indication of population structure among the offshore animals, it would be advantageous to collect biopsy samples from dolphins located between the putative Off-SCB and Off-SD populations, as well as outside of the Southern California Bight area. This would contribute to a better understanding of the population structure among the offshore animals in the ENP region.

To clarify the southern range of the coastal population, samples should be collected along the Mexican coast, particularly at Ensenada and San Quintín where photo-identification studies have indicated only one percent of identified coastal dolphins off San Quintín matched coastal dolphins identified within the range of Ensenada to Santa Barbara, California (Caldwell 1992; Defran et al. 1999). Also, to get a better understanding of the northern movement of this population, biopsies should be obtained north of the San Diego region to determine if the findings of this study persist with increased sample size from other coastal locations along California.

Since the coastal and offshore ecotypes in the ENP are indicated to be distinct populations, it is important to learn more about each form's life history parameters and morphology. To date, only one study was conducted on the ENP ecotypes and it was with small sample sizes (Walker 1981). More effective and

applicable management efforts can be enforced with increased knowledge on the similarities and differences of these dolphin ecotypes in the ENP.

Future research should include coastal and offshore genetic studies in other locations where the two ecotypes of bottlenose dolphins occur and in turn, compared to the results presented here. It would also be beneficial to conduct a genetic analysis on bottlenose dolphins of the Eastern Tropical Pacific to further evaluate population structure within the eastern North Pacific Ocean and compare to the results of the current study. A global, comprehensive analysis of bottlenose dolphins would provide a more complete examination of phylogeographical and phylogenetic relationships between and within ocean basins. The discovery of global trends would contribute information of the nominal species to the speciation debate within the genus *Tursiops*.

CHAPTER 6: CONCLUSIONS

The two ecotypes of bottlenose dolphins (*Tursiops truncatus*), coastal and offshore, identified in the eastern North Pacific region were found to be genetically distinct populations. Previously distinguished by morphological and photo-identification studies, these forms are currently managed as separate stocks and this genetic study supports that designation. The degree of genetic differentiation indicated the coastal animals to have had reduced gene flow from the offshore, ETP, and Gulf of California populations within the ENP region for some time. Diligent monitoring of health and water quality for the coastal population should begin as they are a small population susceptible to harmful anthropogenic and land run-off influences due to their narrow 'coastal corridor' habitat.

The data also suggested population structure among the offshore dolphins as the southern offshore group (Off-SD) was less divergent from the ETP and Gulf-CA populations than the northern offshore group (Off-SCB). The fact that such strong genetic differentiation was found between the populations of the four ENP areas of this study using small sample sizes suggests evident population structure. Further sampling will be necessary to determine the extent of structure in this region.

The results of this study are in concordance with the findings of other global *T. truncatus* genetic studies where coastal and offshore animals were found to be distinct, with the coastal animals having lower genetic variation. Comparing these results to studies of coastal and offshore populations in the WNA, the

degree of genetic distinctness was higher in the WNA. This greater genetic differentiation may be contributed by key differences between the coasts such as habitat, oceanography, and population size. Future research should include coastal and offshore genetic studies in other locations within the *Tursiops* distributions, where the two forms have been identified, to compare those results with the ones collected in this study of the eastern North Pacific, off the western United States.

LITERATURE CITED

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APPENDIX I: *T. truncatus* samples

SWFSC Archive #	Location	Gender	Haplotype #	Collection Date	Sample Type
Offshore CA					
1300	Oregon	F	1	21-Sep-92	Fishery gillnet
4495	Off-SCB	M	2	18-Aug-95	Biopsy
4496	Off-SCB	F	2	18-Aug-95	Biopsy
4498	Off-SCB	F	3	18-Aug-95	Biopsy
5814	Off-SCB	M	4	1-Aug-96	Biopsy
5815	Off-SCB	F	5	1-Aug-96	Biopsy
5816	Off-SCB	F	6	1-Aug-96	Biopsy
5817	Off-SCB	F	6	2-Aug-96	Biopsy
5818	Off-SCB	F	7	2-Aug-96	Biopsy
6151	Off-SCB	M	8	5-Sep-96	Biopsy
6153	Off-SCB	M	8	5-Sep-96	Biopsy
6290	Off-SCB	M	9	13-Oct-96	Biopsy
18650	Off-SD	F	5	22-Dec-00	Biopsy
18651	Off-SD	F	10	22-Dec-00	Biopsy
18652	Off-SD	M	11	22-Dec-00	Biopsy
18653	Off-SD	M	12	22-Dec-00	Biopsy
18654	Off-SD	F	13	22-Dec-00	Biopsy
18655	Off-SD	M	14	22-Dec-00	Biopsy
23792	Off-SD	F	15	8-Jun-01	Biopsy
23793	Off-SD	F	8	8-Jun-01	Biopsy
23794	Off-SD	M	16	8-Jun-01	Biopsy
23801	Off-SD	F	8	10-Jun-01	Biopsy
25182	Off-SD	M	17	6-Oct-01	Biopsy
25184	Off-SD	M	18	6-Oct-01	Biopsy
25185	Off-SD	M	19	6-Oct-01	Biopsy
25186	Off-SD	M	13	6-Oct-01	Biopsy
25469	Off-SCB	M	14	2-Oct-01	Biopsy
25470	Off-SCB	M	20	2-Oct-01	Biopsy
25471	Off-SCB	F	8	2-Oct-01	Biopsy
26304	Off-SCB	F	21	9-Nov-01	Biopsy
26305	Off-SCB	M	22	9-Nov-01	Biopsy
26310	Off-SCB	F	8	9-Nov-01	Biopsy
26316	Off-SCB	F	23	9-Nov-01	Biopsy
26317	Off-SCB	F	7	9-Nov-01	Biopsy
26318	Off-SCB	M	3	9-Nov-01	Biopsy
26320	Off-SCB	M	7	9-Nov-01	Biopsy
31888	Off-SD	M	24	27-Oct-02	Biopsy
41757	Off-SD	M	19	18-Aug-04	Biopsy
41758	Off-SD	F	25	18-Aug-04	Biopsy
41759	Off-SD	M	8	18-Aug-04	Biopsy

SWFSC Archive #	Location	Gender	Haplotype #	Collection Date	Sample Type
Coastal CA					
23945	La Jolla	M	26	22-Jun-01	Biopsy
25503	La Jolla	M	27	11-Nov-01	Biopsy
25509	Mission Bay	F	27	11-Nov-01	Biopsy
40915	Torrey Pines	M	28	7-Jul-04	Biopsy
40916	Torrey Pines	M	28	7-Jul-04	Biopsy
41538	Torrey Pines	F	28	3-Aug-04	Biopsy
41539	Torrey Pines	M	29	3-Aug-04	Biopsy
41540	Torrey Pines	M	28	3-Aug-04	Biopsy
41578	Torrey Pines	F	29	11-Aug-04	Biopsy
41579	Torrey Pines	F	28	11-Aug-04	Biopsy
41819	Del Mar	M	27	28-Sep-04	Biopsy
41820	Carlsbad	M	27	28-Sep-04	Biopsy
41821	Carlsbad	F	29	28-Sep-04	Biopsy
41822	Carlsbad	M	27	28-Sep-04	Biopsy
42192	Leucadia	M	29	22-Oct-04	Biopsy
42193	Leucadia	M	27	22-Oct-04	Biopsy
44718	Blacks Beach	M	29	4-Feb-05	Biopsy
44719	Torrey Pines	M	27	4-Feb-05	Biopsy
44720	Torrey Pines	M	28	4-Feb-05	Biopsy
44721	Torrey Pines	M	29	4-Feb-05	Biopsy
45229	La Jolla	F	27	1-Feb-05	Biopsy
45230	La Jolla	F	27	1-Feb-05	Biopsy
45231	La Jolla	M	29	1-Feb-05	Biopsy
45232	La Jolla	F	29	1-Feb-05	Biopsy
45233	La Jolla	M	26	1-Feb-05	Biopsy
45234	La Jolla	M	30	1-Feb-05	Biopsy
45236	Torrey Pines	M	29	8-Feb-05	Biopsy
45237	Blacks Beach	F	30	8-Feb-05	Biopsy
49095	La Jolla	F	26	7-Sep-05	Biopsy
Gulf of CA					
1712	Offshore	M	7	14-Aug-93	Biopsy
13436	Coastal	F	1	19-Aug-97	Biopsy
13440	Coastal	F	1	27-Aug-97	Biopsy
13441	Coastal	F	1	27-Aug-97	Biopsy
13442	Coastal	F	1	27-Aug-97	Biopsy
13452	Coastal	F	2	8-Sep-97	Biopsy
13454	Coastal	M	19	15-Sep-97	Biopsy
Offshore ETP					
748	ETP	M	31	26-Jun-92	Fishery
749	ETP	M	31	26-Jun-92	Fishery
750	ETP	F	36	26-Jun-92	Fishery
1164	Central America	?	37	21-Aug-92	Biopsy
1935	Baja CA, Mexico	F	35	31-Oct-93	Biopsy
18490	ETP	F	31	28-Nov-00	Biopsy

SWFSC Archive #	Location	Gender	Haplotype #	Collection Date	Sample Type
18491	ETP	F	32	28-Nov-00	Biopsy
18522	Baja CA, Mexico	M	33	6-Dec-00	Biopsy
18523	Baja CA, Mexico	M	12	6-Dec-00	Biopsy
18524	Baja CA, Mexico	M	34	6-Dec-00	Biopsy

APPENDIX II: Sighting data source

SWFSC Cruise Survey	Year
ETP-Porp Density Survey	1974
ETP-SOPS	1976-77, 1979-80
ETP-Behavior	1976
ETP-Calib/Avoidance	1976
ETP-Equatorial Front	1977
ETP-Baitboat	1978
ETP	1978
ETP-SOPS	1979-80
California Current	1980
ETP-EPOCS	1980-81
CA	1982
ETP-PPAS	1982-83
ETP-Avoidance	1983
CA	1983
ETP-MOPS	1986-90
ETP-CAMMS	1991
ETP-PODS	1992
CA/MX-PODS	1993
Gulf CA-CADDIS	1995
CA/OR/WA-ORCAWALE	1996, 2001

SWFSC Cruise Survey	**Year**
NE Pacific-SWAPS	1997
ETP-SPAM	1998
ETP-STAR	1999-2000
Hawaii-HICEAS	2002
ETP-STAR	2003
Aerial Survey	**Year**
CA Coast-CCS1991	1991
CA Coast-CCS1992	1992
San Nicolas Island-NVY1993	1993
San Nicolas Island-NVY1994	1994
San Clemente Island-NVY1998	1998
San Clemente Island-NVY1999	1999
San Clemente Island-NVY2001	2001